PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

# PCT

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

| | |
|---|---|
| **Date of mailing** (day/month/year)<br>07 May 2001 (07.05.01) | |
| **International application No.**<br>PCT/GB00/03159 | **Applicant's or agent's file reference**<br>PC/GOW/P10890PC |
| **International filing date** (day/month/year)<br>16 August 2000 (16.08.00) | **Priority date** (day/month/year)<br>18 August 1999 (18.08.99) |
| **Applicant**<br>GALBRAITH, Daniel et al | |

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

09 March 2001 (09.03.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

| | |
|---|---|
| The International Bureau of WIPO<br>34, chemin des Colombettes<br>1211 Geneva 20, Switzerland | Authorized officer<br>Juan Cruz |
| Facsimile No.: (41-22) 740.14.35 | Telephone No.: (41-22) 338.83.38 |

PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

| Applicant's or agent's file reference | FOR FURTHER ACTION | see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below. |
|---|---|---|
| PC/GOW/P10890PC | | |
| International application No. | International filing date *(day/month/year)* | (Earliest) Priority Date *(day/month/year)* |
| PCT/GB 00/ 03159 | 16/08/2000 | 18/08/1999 |
| Applicant | | |
| QUIP TECHNOLOGY LIMITED | | |

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 8 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority in written form.

   ☒ furnished subsequently to this Authority in computer readble form.

   ☒ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☒ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   ☐ the text is approved as submitted by the applicant.

   ☒ the text has been established by this Authority to read as follows:

   PORCINE ENDOVIRUS GAG AND ENV AND DIAGNOSTIC USES THEREOF

5. With regard to the **abstract,**

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

   ☐ as suggested by the applicant.

   ☒ because the applicant failed to suggest a figure.

   ☐ because this figure better characterizes the invention.

   ☐ None of the figures.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

A porcine endogenous retrovirus (PoERV) ENV polypeptide fragment, wherein said ENV polypeptide fragment comprises the amino acid sequence SEQ ID NO 15 identified as Peptide E ; a porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises said fragment Peptide E and a fragment of a PoERV GAG polypeptide; an antiserum specific to a said Peptide ED; a specific antibody of fragment thereof raised against said Peptide E and use thereof in the detection of PoERV in a sample; uses of said Peptide E or said specific antibody in the detection of PoERV in a sample or in therapy ; an assay kit for use in the detection of PoERV in a sample comprising either said Peptide E or said specific antibody;

5. Claim : Partially 1 and 6 to 19

A porcine endogenous retrovirus (PoERV) ENV polypeptide fragment, wherein said ENV polypeptide fragment comprises the amino acid sequence SEQ ID NO 16 identified as Peptide F ; a porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises said fragment Peptide F and a fragment of a PoERV GAG polypeptide; an antiserum specific to a said Peptide F; a specific antibody of fragment thereof raised against said Peptide F and use thereof in the detection of PoERV in a sample; uses of said Peptide F or said specific antibody in the detection of PoERV in a sample or in therapy ; an assay kit for use in the detection of PoERV in a sample comprising either said Peptide F or said specific antibody;

6. Claim : Partially 1 and 6 to 19

A porcine endogenous retrovirus (PoERV) ENV polypeptide fragment, wherein said ENV polypeptide fragment comprises the amino acid sequence SEQ ID NO 17 identified as Peptide G ; a porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises said fragment Peptide G and a fragment of a PoERV GAG polypeptide; an antiserum specific to a said Peptide G; a specific antibody of fragment thereof raised against said Peptide G and use thereof in the detection of PoERV in a sample; uses of said Peptide G or said specific antibody in the detection of PoERV in a sample or in therapy ; an assay kit for use in the detection of PoERV in a sample comprising either said Peptide G or said specific antibody;

7. Claim : Partially 1 and 6 to 19

A porcine endogenous retrovirus (PoERV) ENV polypeptide fragment, wherein said ENV polypeptide fragment comprises the amino acid sequence SEQ ID NO 18 identified as Peptide H

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

; a porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises said fragment Peptide H and a fragment of a PoERV GAG polypeptide; an antiserum specific to a said Peptide H; a specific antibody of fragment thereof raised against said Peptide H and use thereof in the detection of PoERV in a sample; uses of said Peptide H or said specific antibody in the detection of PoERV in a sample or in therapy ; an assay kit for use in the detection of PoERV in a sample comprising either said Peptide H or said specific antibody;

8. Claim : Partially 1 and 6 to 19

A porcine endogenous retrovirus (PoERV) ENV polypeptide fragment, wherein said ENV polypeptide fragment comprises the amino acid sequence SEQ ID NO 19 identified as Peptide J ; a porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises said fragment Peptide J and a fragment of a PoERV GAG polypeptide; an antiserum specific to a said Peptide J; a specific antibody of fragment thereof raised against said Peptide J and use thereof in the detection of PoERV in a sample; uses of said Peptide J or said specific antibody in the detection of PoERV in a sample or in therapy ; an assay kit for use in the detection of PoERV in a sample comprising either said Peptide J or said specific antibody;

9. Claim : Partially 12 to 19

A porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises a fragment of a PoERV GAG polypeptide and a fragment of a PoERV ENV polypeptide, as far as not covered by a previous subject; an antiserum specific to a said PoERV polypeptide; a specific antibody of fragment thereof raised against said PoERV polypeptide and use thereof in the detection of PoERV in a sample; uses of said PoERV polypeptide or said specific antibody in the detection of PoERV in a sample or in therapy ; an assay kit for use in the detection of PoERV in a sample comprising either said PoERV polypeptide or said specific antibody.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 00/03159

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sh t)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 19 is partially directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

see extra sheet invention 1.

**Remark on Prot st**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claim : 4 and partially 1 to 3 and 12 to 19

A porcine endogenous retrovirus (PoERV) GAG polypeptide fragment, wherein said GAG polypeptide fragment comprises the amino acid sequence SEQ ID NO 12 identified as Peptide 1; a porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises said fragment Peptide 1 and a fragment of a PoERV ENV polypeptide; an antiserum specific to a said Peptide 1; a specific antibody of fragment thereof raised against said Peptide 1 and use thereof in the detection of PoERV in a sample; uses of said Peptide 1 or said specific antibody in the detection of PoERV in a sample or in therapy ; an assay kit for use in the detection of PoERV in a sample comprising either said Peptide 1 or said specific antibody;

2. Claim : 5 and partially 1 to 3 and 12 to 19

A porcine endogenous retrovirus (PoERV) GAG polypeptide fragment, wherein said GAG polypeptide fragment comprises the amino acid sequence SEQ ID NO 13 identified as Peptide 2; a porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises said fragment Peptide 2 and a fragment of a PoERV ENV polypeptide; an antiserum specific to a said Peptide 2; a specific antibody of fragment thereof raised against said Peptide 2 and use thereof in the detection of PoERV in a sample; uses of said Peptide 2 or said specific antibody in the detection of PoERV in a sample or in therapy ; an assay kit for use in the detection of PoERV in a sample comprising either said Peptide 2 or said specific antibody;

3. Claim : Partially 1 and 6 to 19

A porcine endogenous retrovirus (PoERV) ENV polypeptide fragment, wherein said ENV polypeptide fragment comprises the amino acid sequence SEQ ID NO 14 identified as Peptide D ; a porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises said fragment Peptide D and a fragment of a PoERV GAG polypeptide; an antiserum specific to a said Peptide D; a specific antibody of fragment thereof raised against said Peptide D and use thereof in the detection of PoERV in a sample; uses of said Peptide D or said specific antibody in the detection of PoERV in a sample or in therapy ; an assay kit for use in the detection of PoERV in a sample comprising either said Peptide D or said specific antibody;

4. Claim : Partially 1 and 6 to 19

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 00/03159

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/49 C07K14/15 G01N33/50 C07K16/10 A61K39/21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | AKIYOSHI D E ET AL: "IDENTIFICATION OF A FULL-LENGTH CDNA FOR AN ENDOGENOUS RETROVIRUS OF MINIATURE SWINE" JOURNAL OF VIROLOGY, THE AMERICAN SOCIETY FOR MICROBIOLOGY, US, vol. 72, no. 5, May 1998 (1998-05), pages 4503-4507, XP001002575 ISSN: 0022-538X page 4504, column 2, paragraph 2; table 1 page 4507, paragraph 2 - paragraph 3 --- -/-- | 1-4,19 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 16 August 2001 | 31. 10. 2001 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | CHAMBONNET, F |

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | MATTHEWS A L ET AL: "DEVELOPMENT AND VALIDATION OF A WESTERN IMMUNOBLOT ASSAY FOR DETECTION OF ANTIBODIES TO PORCINE ENDOGENOUS RETROVIRUS" TRANSPLANTATION, WILLIAMS AND WILKINS, BALTIMORE, MD, US, vol. 67, no. 7, 15 April 1999 (1999-04-15), pages 939-943, XP001006259 ISSN: 0041-1337 cited in the application the whole document | 1,2, 13-16,19 |
| X | WO 97 40167 A (GALBRAITH DANIEL NORMAN ;HAWORTH CHRISTINE (GB); LEES GILLIAN MARG) 30 October 1997 (1997-10-30) cited in the application page 3; claims 13-18,27,29,30; figure 3 | 1-4,12, 19 |
| P,X | GALBRAITH DN, KELLY HT, DYKE A, REID G, HAWORTH C, BEEKMAN J, SHEPHERD A, SMITH KT.: "Design and validation of immunological tests for the detection of Porcine endogenous retrovirus in biological materials." J VIROL METHODS. 2000 NOV;90(2):115-24., XP001006236 the whole document | 1-4, 13-19 |
| P,A | WILSON C A ET AL: "EXTENDED ANALYSIS OF THE IN VITRO TROPISM OF PORCINE ENDOGENOUS RETROVIRUS" JOURNAL OF VIROLOGY, THE AMERICAN SOCIETY FOR MICROBIOLOGY, US, vol. 74, no. 1, January 2000 (2000-01), pages 49-56, XP001002577 ISSN: 0022-538X | |
| P,A | BLUSCH J H ET AL: "A POLYMERASE CHAIN REACTION-BASED PROTOCOL FOR THE DETECTION OF TRANSMISSION OF PIG ENDOGENOUS RETROVIRUSES IN PIG TO HUMAN XENOTRANSPLANTATION" TRANSPLANTATION, WILLIAMS AND WILKINS, BALTIMORE, MD, US, vol. 69, no. 10, 27 May 2000 (2000-05-27), pages 2176-2172, XP001005967 ISSN: 0041-1337 | |

2

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 00/03159

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 9740167 A | 30-10-1997 | AU 721579 B | 06-07-2000 |
| | | AU 2394697 A | 12-11-1997 |
| | | CA 2251939 A | 30-10-1997 |
| | | EP 0907739 A | 14-04-1999 |
| | | JP 2000512129 T | 19-09-2000 |

# ATENT COOPERATION TR[redacted]TY

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
MCCALLUM, William Potter
CRUIKSHANK & FAIRWEATHER
19 Royal Exchange Square
Glasgow G1 3AE
GRANDE BRETAGNE

# PCT

## WRITTEN OPINION

(PCT Rule 66)

| | |
|---|---|
| Date of mailing *(day/month/year)* | 13.12.2001 |
| Applicant's or agent's file reference: PC/SJB/P10890PC | **REPLY DUE** **within 1 month(s)** from the above date of mailing |

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
|---|---|---|
| PCT/GB00/03159 | 16/08/2000 | 18/08/1999 |

International Patent Classification (IPC) or both national classification and IPC
C12N15/49

Applicant
QUIP TECHNOLOGY LIMITED et al.

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

| | | |
|---|---|---|
| I | ☒ | Basis of the opinion |
| II | ☐ | Priority |
| III | ☒ | Non-establishment of opinion with regard to novelty, inventive step and industrial applicability |
| IV | ☒ | Lack of unity of invention |
| V | ☒ | Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement |
| VI | ☐ | Certain document cited |
| VII | ☐ | Certain defects in the international application |
| VIII | ☐ | Certain observations on the international application |

3. The applicant is hereby **invited to reply** to this opinion.

**When?** See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

**How?** By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

**Also:** For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

**If no reply is filed,** the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 18/12/2001.

Name and mailing address of the international preliminary examining authority:
European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer / Examiner
Mundel, C

Formalities officer (incl. extension of time limits)
Zoglauer, H
Telephone No. +49 89 2399 8051

## WRITTEN OPINION

International application No. PCT/GB00/03159

### I. Basis of the opinion

1. With regard to the **elements** of the international application (Replacement *sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed"*):

**Description, pages:**

| | | | | |
|---|---|---|---|---|
| 1-13,15-35 | as originally filed | | | |
| 14 | as received on | 25/10/2000 | with letter of | 17/10/2000 |

**Claims, No.:**

| | |
|---|---|
| 1-19 | as originally filed |

**Drawings, sheets:**

| | | | | |
|---|---|---|---|---|
| 1/4-4/4 | as received on | 25/10/2000 | with letter of | 17/10/2000 |

**Sequence listing part of the description, pages:**

1-21 (phoenix : 2-22), filed with the letter of 03.11.00

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:
   ☐ the claims, Nos.:
   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   *(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 5-11 (completely) and 1-3 and 12-19 (partially),

because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 5-11 (completely) and 1-3 and 12-19 (partially).

2. A written opinion cannot be drawn due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.
   ☐ the computer readable form has not been furnished or does not comply with the standard.

**IV. Lack of unity of invention**

## WRITTEN OPINION

International application No. PCT/GB00/03159

1. In response to the invitation (Form PCT/IPEA/405) to restrict or pay additional fees, the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with for the following reasons and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees:
   **see separate sheet**

3. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this opinion:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 4 (completely) and 1-3 and 12-19 (partially).

**V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | |
|---|---|---|
| Novelty (N) | Claims | 1-4, 13-14, 16 and 18-19 (NO) |
| Inventive step (IS) | Claims | 1-4 and 12-19 (NO) |
| Industrial applicability (IA) | Claims | |

2. Citations and explanations
   **see separate sheet**

Re Item III

**Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

A lack of unity objection was raised by the International Search Autority (ISA) for the present application (see also point IV below). Since no additional fees were paid, the only invention which has been searched, and examined, is the invention 1.

Re Item IV

**Lack of unity of invention**

According to **Rule 13 PCT** an application must relate only to one invention or to a group of inventions so linked as to form a **single inventive concept**, i.e. having at least one common technical feature defining a contribution over the known prior art.

The IPEA agrees with the ISA advices and identifies the following groups of inventions.

1. Claims 4 (completely) and 1-3 and 12-19 (partially)

   A porcine endogenous retrovirus (PoERV) GAG polypeptide fragment, wherein said GAG polypeptide fragment comprises the amino acid sequence SEQ ID NO:12 identified as peptide 1; a porcine endogenous retrovirus (PoERV) polypeptide fragment wherein said fragment comprises said peptide 1 and a fragment of a PoERV ENV polypeptide; an antiserum specific to said peptide 1; a specific antibody or fragment thereof raised against said peptide 1 and uses thereof in the detection of PoERV in a sample; uses of said peptide 1 or said specific antibody in the detection of PoERV in a sample or in therapy; an assay kit for use in the detection of PoERV in a sample comprising either said peptide 1 or said specific antibody.

2. Claims 5 (completely) and 1-3 and 12-19 (partially).

   A porcine endogenous retrovirus (PoERV) GAG polypeptide fragment, wherein said GAG polypeptide fragment comprises the amino acid sequence SEQ ID NO:13 identified as peptide 2; a porcine endogenous retrovirus (PoERV)

polypeptide fragment wherein said fragment comprises said peptide 2 and a fragment of a PoERV ENV polypeptide; an antiserum specific to said peptide 2; a specific antibody or fragment thereof raised against said peptide 2 and uses thereof in the detection of PoERV in a sample; uses of said peptide 2 or said specific antibody in the detection of PoERV in a sample or in therapy; an assay kit for use in the detection of PoERV in a sample comprising either said peptide 2 or said specific antibody.

3. Claims 1 and 6-19 (partially)

A porcine endogenous retrovirus (PoERV) ENV polypeptide fragment, wherein said ENV polypeptide fragment comprises the amino acid sequence SEQ ID NO:14 identified as peptide D; a porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises said fragment peptide D and a fragment of a PoERV GAG polypeptide; an antiserum specific to said peptide D; a specific antibody or fragment thereof raised against said peptide D and use thereof in the detection of PoERV in a sample; uses of said peptide D or said specific antibody in the detection of PoERV in a sample or in therapy; an assay kit for use in the detection of PoERV in a sample comprising either said peptide D or said specific antibody.

4. Claims 1 and 6-19 (partially)

A porcine endogenous retrovirus (PoERV) ENV polypeptide fragment, wherein said ENV polypeptide fragment comprises the amino acid sequence SEQ ID NO:15 identified as peptide E; a porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises said fragment peptide E and a fragment of a PoERV GAG polypeptide; an antiserum specific to said peptide E; a specific antibody or fragment thereof raised against said peptide E and use thereof in the detection of PoERV in a sample; uses of said peptide E or said specific antibody in the detection of PoERV in a sample or in therapy; an assay kit for use in the detection of PoERV in a sample comprising either said peptide E or said specific antibody.

5. Claims 1 and 6-19 (partially)

A porcine endogenous retrovirus (PoERV) ENV polypeptide fragment, wherein said ENV polypeptide fragment comprises the amino acid sequence SEQ ID NO:16 identified as peptide F; a porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises said fragment peptide F and a fragment of a PoERV GAG polypeptide; an antiserum specific to said peptide F; a specific antibody or fragment thereof raised against said peptide F and use thereof in the detection of PoERV in a sample; uses of said peptide F or said specific antibody in the detection of PoERV in a sample or in therapy; an assay kit for use in the detection of PoERV in a sample comprising either said peptide F or said specific antibody.

6. Claims 1 and 6-19 (partially)

A porcine endogenous retrovirus (PoERV) ENV polypeptide fragment, wherein said ENV polypeptide fragment comprises the amino acid sequence SEQ ID NO:17 identified as peptide G; a porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises said fragment peptide G and a fragment of a PoERV GAG polypeptide; an antiserum specific to said peptide G; a specific antibody or fragment thereof raised against said peptide G and use thereof in the detection of PoERV in a sample; uses of said peptide G or said specific antibody in the detection of PoERV in a sample or in therapy; an assay kit for use in the detection of PoERV in a sample comprising either said peptide G or said specific antibody.

7. Claims 1 and 6-19 (partially)

A porcine endogenous retrovirus (PoERV) ENV polypeptide fragment, wherein said ENV polypeptide fragment comprises the amino acid sequence SEQ ID NO:18 identified as peptide H; a porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises said fragment peptide H and a fragment of a PoERV GAG polypeptide; an antiserum specific to said peptide H; a specific antibody or fragment thereof raised against said peptide H and use thereof in the detection of PoERV in a sample; uses of said peptide H or said specific antibody in the detection of PoERV in a sample or in therapy; an assay kit for use in the detection of PoERV in a sample comprising either said

peptide H or said specific antibody.

8. Claims 1 and 6-19 (partially)

A porcine endogenous retrovirus (PoERV) ENV polypeptide fragment, wherein said ENV polypeptide fragment comprises the amino acid sequence SEQ ID NO:19 identified as peptide J; a porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises said fragment peptide J and a fragment of a PoERV GAG polypeptide; an antiserum specific to said peptide J; a specific antibody or fragment thereof raised against said peptide J and use thereof in the detection of PoERV in a sample; uses of said peptide J or said specific antibody in the detection of PoERV in a sample or in therapy; an assay kit for use in the detection of PoERV in a sample comprising either said peptide J or said specific antibody.

9. Claim 12-19 (partially)

A porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said fragment comprises a fragment of a PoERV GAG polypeptide and a fragment of a PoERV ENV polypeptide, as far as not covered by a previous subject; an antiserum specific to said PoERV polypeptide; a specific antibody or fragment thereof raised against said PoERV polypeptide and use thereof in the detection of PoERV in a sample; uses of said PoERV polypeptide or said specific antibody in the detection of PoERV in a sample or in therapy; an assay kit for use in the detection of PoERV in a sample comprising either said PoERV polypeptide or aid specific antibody.

The document Transplantation, vol. 67, issue 7, pp. 939-943 published on April 15th 1999 : "Development and validation of a Western immunoblot assay for detection of antibodies to porcine endogenous retrovirus", from Matthews A.L. et al., already reported the development and validation of a PoERV-specific Western immunoblot assay for the diagnostic testing of porcine xenografts recipients. A serological assay was disclosed for detecting antibodies to the GAG proteins of PoERV, and particularly with the 30 kDa (major) protein present in PK-15 cells and in the PoERV-infected 293 human kidney cells.

Moreover, the document Journal of Virology, vol. 72(5), pp. 4503-4507 (May 1999) : "Identification of a full-length cDNA for an endogenous retrovirus of miniature swine", from Akiyoshi D.E. et al., already characterized the nucleotide sequences of porcine endogenous retrovirus taken from lymphocytes of miniature swine (PERV-MSL) (accession number AF038600), Tsukuba-1 retrovirus (accession number AF038601), and PK15 (PK15-ERV) (accession number AF038599) (cf p. 4507, column 1, last paragraph) and compared the predicted amino acid sequences of the different ORFs (p. 4504, column 2, Table 1 and paragraph 2 and 4).

Therefore, a porcine endogenous retrovirus (PoERV) GAG polypeptide fragment, wherein said GAG polypeptide fragment has PoERV specific antigenic or immunogenic activity and comprising fragment of the polypeptide sequence shown on Fig. 1 of the underlying application, is already known and suitable for a serological assay to detect PoERV in a sample.

In view of this prior art, the general problem of the underlying application is to provide further serological assays for the detection of PoERV in a sample

* A first general described solution provides further porcine endogenous retrovirus (PoERV) GAG polypeptide fragments :
- a first porcine endogenous retrovirus (PoERV) GAG polypeptide fragment comprising the amino acid sequence SEQ ID NO:12 of the underlying application and identified as "peptide 1"
- a second porcine endogenous retrovirus (PoERV) GAG polypeptide fragment comprising the amino acid sequence SEQ ID NO:13 of the underlying application and identified as "peptide 2".

* A second general described solution provides further porcine endogenous retrovirus (PoERV) ENV polypeptide fragments :
- a porcine endogenous retrovirus (PoERV) ENV polypeptide fragment comprising the amino acid sequence SEQ ID NO:14 of the underlying application and identified as "peptide D".
- a porcine endogenous retrovirus (PoERV) ENV polypeptide fragment comprising the amino acid sequence SEQ ID NO:15 of the underlying application and identified as "peptide E".

- a porcine endogenous retrovirus (PoERV) ENV polypeptide fragment comprising the amino acid sequence SEQ ID NO:16 of the underlying application and identified as "peptide F".
- a porcine endogenous retrovirus (PoERV) ENV polypeptide fragment comprising the amino acid sequence SEQ ID NO:17 of the underlying application and identified as "peptide G".
- a porcine endogenous retrovirus (PoERV) ENV polypeptide fragment comprising the amino acid sequence SEQ ID NO:18 of the underlying application and identified as "peptide H".

a porcine endogenous retrovirus (PoERV) ENV polypeptide fragment comprising the amino acid sequence SEQ ID NO:19 of the underlying application and identified as "peptide J".

In view of the fact that antigenic and immunogenic porcine endogenous retrovirus (PoERV) polypeptide fragments are already known as well as their use for a serological assay to detect PoERV in a sample, considering the essential differences in amino acid sequence of the described solutions and due to the fact that no other technical features can be distinguished which, in the light of the prior art, could be regarded as special technical features, the IPEA agree with the ISA advice that there is no single inventive concept underlying the plurality of claimed inventions of the present underlying application in the sense of rule 131 PCT. Consequently, there is a lack of unity and the different inventions, not belonging to a common inventive concept, are considered as independent inventions.

**Re Item V**

**Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. The present application refers to a fragment of the GAG polypeptide of a porcine endogenous retrovirus (PoERV) comprising the amino acid sequence REERRDRRQEKNLTK and having a PoERV specific antigenic or immunogenic activity, to such a polypeptide comprising additionally a fragment of the PoERV ENV polypeptide, to an antiserum or an antibody specific to such a polypeptide fragment, and to the therapeutical or diagnostic use of said polypeptide fragments or antibodies.

**2. Reference is made to the following documents :**

D1: AKIYOSHI D E ET AL: 'IDENTIFICATION OF A FULL-LENGTH CDNA FOR AN ENDOGENOUS RETROVIRUS OF MINIATURE SWINE' JOURNAL OF VIROLOGY, THE AMERICAN SOCIETY FOR MICROBIOLOGY, US, vol. 72, no. 5, May 1998 (1998-05), pages 4503-4507.

D2: MATTHEWS A L ET AL: 'DEVELOPMENT AND VALIDATION OF A WESTERN IMMUNOBLOT ASSAY FOR DETECTION OF ANTIBODIES TO PORCINE ENDOGENOUS RETROVIRUS' TRANSPLANTATION, WILLIAMS AND WILKINS, BALTIMORE, MD, US, vol. 67, no. 7, 15 April 1999 (1999-04-15), pages 939-943, cited in the application

D3: WO 97 40167 A (GALBRAITH DANIEL NORMAN ;HAWORTH CHRISTINE (GB); LEES GILLIAN MARG) 30 October 1997 (1997-10-30) cited in the application

**3. Lack of novelty; article 33(2) PCT.**

3.1 The document D2 discloses the development and the validation of a western immunoblot assay for the detection of antibodies to porcine endogenous retrovirus (Title). This test was developed for the diagnostic testing of porcine xenografts recipients (p. 939, left-hand column, lines 5-8). The polypeptide used for the test in D2 is the GAG polypeptide of the PoERV which comprises the amino acid sequence REERRDRRQEKNLTK. Moreover, said polypeptide has a PoERV specific antigenic or immunogenic activity.
The attention of the applicant is drawn to the fact that the authors of D2 were aware that the nucleotide sequence of a PoERV was already known (p. 940, left-hand column, lines 49-59).

Therefore, the subject-matter of claims 1-4, 13, 16, 18 and 19 can not be considered as novel in the sense of article 33(2) PCT.

3.2 The document D1 discloses the identification of a full-length cDNA of an endogenous retrovirus of miniature swine. According to the authors of D1,

the existence of PoERV clones will enable studies of infection by endogenous retroviruses in xenotransplantation (p. 4503, Abstract, lines 10-11). Table 1 of D1 (p. 4504) shows the identities between the GAG, POL and ENV polypeptides of PERV-MSL and the corresponding polypeptides of the porcine endogenous retroviruses tsukuba-1 and PK15-ERV.

Therefore, the subject-matter of claims 1-4 can not be considered as novel in the sense of article 33(2) PCT.

3.3 The document D3 discloses, inter alia, porcine retrovirus (PoEV) polypeptides, antibodies immuno-reactive with PoEV polypeptides, pharmaceutical compositions comprising recombinant PoEV polypeptides for use as prophylactic and/or therapeutic agents and the use of said polypeptides in the preparation of medicament for use in medicine (Abstract). D3 also claims the use of the recombinant polypeptide in the preparation of a vaccine (claim 27), the use of the polypeptide in therapy or diagnosis (claim 29) and the use of the polypeptide in the fabrication of a medicament (claim 30). The nucleotide and protein sequence of the GAG polypeptide of PoEV is given in Fig. 3 (nucleotides 588-2162). The GAG amino acid sequence disclosed in D3 is very similar to the GAG polypeptide of the present application and comprises the sequence REERRDRRQEKNLTK.

The IPEA considers that the GAG polypeptide of the PoEV disclosed in D3 has a PoERV specific antigenic or immunogenic activity.

Therefore, the subject-matter of claims 1-4, 13-14, 16, 18 and 19 can not be considered as novel in the sense of article 33(2) PCT.

## 4. Lack of inventive step; article 33(3) PCT.

The IPEA is the opinion that the skilled person, knowing from D1 and D2 the nucleotide and corresponding amino acid sequences of porcine endogenous retrovirus (PoERV) and the fact that said PoERV may infect human cells, for example in the case of xenografts, would need no inventive activity to consider

using the GAG polypeptide or antibodies directed against said polypeptide for the therapy or diagnostic of an infection with PoERV, especially in the light of D2 which shows that the GAG polypeptide could be used for the detection of antibodies to porcine endogenous retrovirus.

Therefore, the subject-matter of claims 1-4 and 12-19 can not be considered as inventive in the sense of article 33(3) PCT.

For the discussion about inventive step, the attention of the applicant is drawn to the fact that the selection of fragment comprising the amino acid sequence REERRDRRQEKNLTK could only be considered as inventive if motivated by a technical purpose, i.e. a hitherto unknown or unexpected effect due to the selection of said specific sequence. At the moment, the IPEA fails to see such a technical effect.

10/069082

# PATENT COOPERATION TREATY

# PCT



## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference | FOR FURTHER ACTION | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| PC/SJB/P10890PC | | |
| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
| PCT/GB00/03159 | 16/08/2000 | 18/08/1999 |
| International Patent Classification (IPC) or national classification and IPC<br>C12N15/49 | | |
| Applicant<br>QUIP TECHNOLOGY LIMITED et al. | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 13 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 09/03/2001 | 11.02.2002 |
| Name and mailing address of the international preliminary examining authority:<br>European Patent Office<br>D-80298 Munich<br>Tel. +49 89 2399 - 0 Tx: 523656 epmu d<br>Fax: +49 89 2399 - 4465 | Authorized officer<br>Mundel, C<br>Telephone No. +49 89 2399 7314 |

Form PCT/IPEA/409 (cover sheet) (January 1994)

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/03159

## I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
**Description, pages:**

| | | | | |
|---|---|---|---|---|
| 1-13,15-35 | as originally filed | | | |
| 14 | as received on | 25/10/2000 | with letter of | 17/10/2000 |

**Claims, No.:**

| | |
|---|---|
| 1-19 | as originally filed |

**Drawings, sheets:**

| | | | | |
|---|---|---|---|---|
| 1/4-4/4 | as received on | 25/10/2000 | with letter of | 17/10/2000 |

**Sequence listing part of the description, pages:**

1-21 (phoenix : 2-22), filed with the letter of 03.11.00

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   ☐ the language of publication of the international application (under Rule 48.3(b)).
   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.
   ☐ filed together with the international application in computer readable form.
   ☒ furnished subsequently to this Authority in written form.
   ☒ furnished subsequently to this Authority in computer readable form.
   ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:
   ☐ the claims, Nos.:
   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   *(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 5-11 (completely) and 1-3 and 12-19 (partially).

because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 5-11 (completely) and 1-3 and 12-19 (partially).

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.
   ☐ the computer readable form has not been furnished or does not comply with the standard.

**IV. Lack of unity of invention**

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 4 (completely) and 1-3 and 12-19 (partially).

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 12, 15 and 17 |
| | No: | Claims | 1-4, 13-14, 16 and 18-19 |
| Inventive step (IS) | Yes: | Claims | |
| | No: | Claims | 1-4 and 12-19 |
| Industrial applicability (IA) | Yes: | Claims | 1-4 and 12-19 |
| | No: | Claims | |

2. Citations and explanations
   **see separate sheet**

**Re Item III**

**Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

A lack of unity objection was raised by the International Search Authority (ISA) for the present application (see also point IV below). Since no additional fees were paid, the only invention which has been searched, and examined, is the invention 1.

**Re Item IV**

**Lack of unity of invention**

According to **Rule 13 PCT** an application must relate only to one invention or to a group of inventions so linked as to form a **single inventive concept**, i.e. having at least one common technical feature defining a contribution over the known prior art.

The IPEA agrees with the ISA advices and identifies the following groups of inventions.

1. Claims 4 (completely) and 1-3 and 12-19 (partially)

   A porcine endogenous retrovirus (PoERV) GAG polypeptide fragment, wherein said GAG polypeptide fragment comprises the amino acid sequence SEQ ID NO:12 identified as peptide 1; a porcine endogenous retrovirus (PoERV) polypeptide fragment wherein said fragment comprises said peptide 1 and a fragment of a PoERV ENV polypeptide; an antiserum specific to said peptide 1; a specific antibody or fragment thereof raised against said peptide 1 and uses thereof in the detection of PoERV in a sample; uses of said peptide 1 or said specific antibody in the detection of PoERV in a sample or in therapy; an assay kit for use in the detection of PoERV in a sample comprising either said peptide 1 or said specific antibody.

2. Claims 5 (completely) and 1-3 and 12-19 (partially).

   A porcine endogenous retrovirus (PoERV) GAG polypeptide fragment, wherein said GAG polypeptide fragment comprises the amino acid sequence SEQ ID NO:13 identified as peptide 2; a porcine endogenous retrovirus (PoERV)

polypeptide fragment wherein said fragment comprises said peptide 2 and a fragment of a PoERV ENV polypeptide; an antiserum specific to said peptide 2; a specific antibody or fragment thereof raised against said peptide 2 and uses thereof in the detection of PoERV in a sample; uses of said peptide 2 or said specific antibody in the detection of PoERV in a sample or in therapy; an assay kit for use in the detection of PoERV in a sample comprising either said peptide 2 or said specific antibody.

3. Claims 1 and 6-19 (partially)

A porcine endogenous retrovirus (PoERV) ENV polypeptide fragment, wherein said ENV polypeptide fragment comprises the amino acid sequence SEQ ID NO:14 identified as peptide D; a porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises said fragment peptide D and a fragment of a PoERV GAG polypeptide; an antiserum specific to said peptide D; a specific antibody or fragment thereof raised against said peptide D and use thereof in the detection of PoERV in a sample; uses of said peptide D or said specific antibody in the detection of PoERV in a sample or in therapy; an assay kit for use in the detection of PoERV in a sample comprising either said peptide D or said specific antibody.

4. Claims 1 and 6-19 (partially)

A porcine endogenous retrovirus (PoERV) ENV polypeptide fragment, wherein said ENV polypeptide fragment comprises the amino acid sequence SEQ ID NO:15 identified as peptide E; a porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises said fragment peptide E and a fragment of a PoERV GAG polypeptide; an antiserum specific to said peptide E; a specific antibody or fragment thereof raised against said peptide E and use thereof in the detection of PoERV in a sample; uses of said peptide E or said specific antibody in the detection of PoERV in a sample or in therapy; an assay kit for use in the detection of PoERV in a sample comprising either said peptide E or said specific antibody.

5. Claims 1 and 6-19 (partially)

A porcine endogenous retrovirus (PoERV) ENV polypeptide fragment, wherein said ENV polypeptide fragment comprises the amino acid sequence SEQ ID NO:16 identified as peptide F; a porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises said fragment peptide F and a fragment of a PoERV GAG polypeptide; an antiserum specific to said peptide F; a specific antibody or fragment thereof raised against said peptide F and use thereof in the detection of PoERV in a sample; uses of said peptide F or said specific antibody in the detection of PoERV in a sample or in therapy; an assay kit for use in the detection of PoERV in a sample comprising either said peptide F or said specific antibody.

6. Claims 1 and 6-19 (partially)

A porcine endogenous retrovirus (PoERV) ENV polypeptide fragment, wherein said ENV polypeptide fragment comprises the amino acid sequence SEQ ID NO:17 identified as peptide G; a porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises said fragment peptide G and a fragment of a PoERV GAG polypeptide; an antiserum specific to said peptide G; a specific antibody or fragment thereof raised against said peptide G and use thereof in the detection of PoERV in a sample; uses of said peptide G or said specific antibody in the detection of PoERV in a sample or in therapy; an assay kit for use in the detection of PoERV in a sample comprising either said peptide G or said specific antibody.

7. Claims 1 and 6-19 (partially)

A porcine endogenous retrovirus (PoERV) ENV polypeptide fragment, wherein said ENV polypeptide fragment comprises the amino acid sequence SEQ ID NO:18 identified as peptide H; a porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises said fragment peptide H and a fragment of a PoERV GAG polypeptide; an antiserum specific to said peptide H; a specific antibody or fragment thereof raised against said peptide H and use thereof in the detection of PoERV in a sample; uses of said peptide H or said specific antibody in the detection of PoERV in a sample or in therapy; an assay kit for use in the detection of PoERV in a sample comprising either said

peptide H or said specific antibody.

8. Claims 1 and 6-19 (partially)

A porcine endogenous retrovirus (PoERV) ENV polypeptide fragment, wherein said ENV polypeptide fragment comprises the amino acid sequence SEQ ID NO:19 identified as peptide J; a porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises said fragment peptide J and a fragment of a PoERV GAG polypeptide; an antiserum specific to said peptide J; a specific antibody or fragment thereof raised against said peptide J and use thereof in the detection of PoERV in a sample; uses of said peptide J or said specific antibody in the detection of PoERV in a sample or in therapy; an assay kit for use in the detection of PoERV in a sample comprising either said peptide J or said specific antibody.

9. Claim 12-19 (partially)

A porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said fragment comprises a fragment of a PoERV GAG polypeptide and a fragment of a PoERV ENV polypeptide, as far as not covered by a previous subject; an antiserum specific to said PoERV polypeptide; a specific antibody or fragment thereof raised against said PoERV polypeptide and use thereof in the detection of PoERV in a sample; uses of said PoERV polypeptide or said specific antibody in the detection of PoERV in a sample or in therapy; an assay kit for use in the detection of PoERV in a sample comprising either said PoERV polypeptide or aid specific antibody.

The document Transplantation, vol. 67, issue 7, pp. 939-943 published on April 15th 1999 : "Development and validation of a Western immunoblot assay for detection of antibodies to porcine endogenous retrovirus", from Matthews A.L. et al., already reported the development and validation of a PoERV-specific Western immunoblot assay for the diagnostic testing of porcine xenografts recipients. A serological assay was disclosed for detecting antibodies to the GAG proteins of PoERV, and particularly with the 30 kDa (major) protein present in PK-15 cells and in the PoERV-infected 293 human kidney cells.

Moreover, the document Journal of Virology, vol. 72(5), pp. 4503-4507 (May 1999) : "Identification of a full-length cDNA for an endogenous retrovirus of miniature swine", from Akiyoshi D.E. et al., already characterized the nucleotide sequences of porcine endogenous retrovirus taken from lymphocytes of miniature swine (PERV-MSL) (accession number AF038600), Tsukuba-1 retrovirus (accession number AF038601), and PK15 (PK15-ERV) (accession number AF038599) (cf p. 4507, column 1, last paragraph) and compared the predicted amino acid sequences of the different ORFs (p. 4504, column 2, Table 1 and paragraph 2 and 4).

Therefore, a porcine endogenous retrovirus (PoERV) GAG polypeptide fragment, wherein said GAG polypeptide fragment has PoERV specific antigenic or immunogenic activity and comprising fragment of the polypeptide sequence shown on Fig. 1 of the underlying application, is already known and suitable for a serological assay to detect PoERV in a sample.

In view of this prior art, the general problem of the underlying application is to provide further serological assays for the detection of PoERV in a sample

* A first general described solution provides further porcine endogenous retrovirus (PoERV) GAG polypeptide fragments :
- a first porcine endogenous retrovirus (PoERV) GAG polypeptide fragment comprising the amino acid sequence SEQ ID NO:12 of the underlying application and identified as "peptide 1"
- a second porcine endogenous retrovirus (PoERV) GAG polypeptide fragment comprising the amino acid sequence SEQ ID NO:13 of the underlying application and identified as "peptide 2".

* A second general described solution provides further porcine endogenous retrovirus (PoERV) ENV polypeptide fragments :
- a porcine endogenous retrovirus (PoERV) ENV polypeptide fragment comprising the amino acid sequence SEQ ID NO:14 of the underlying application and identified as "peptide D".
- a porcine endogenous retrovirus (PoERV) ENV polypeptide fragment comprising the amino acid sequence SEQ ID NO:15 of the underlying application and identified as "peptide E".

- a porcine endogenous retrovirus (PoERV) ENV polypeptide fragment comprising the amino acid sequence SEQ ID NO:16 of the underlying application and identified as "peptide F".
- a porcine endogenous retrovirus (PoERV) ENV polypeptide fragment comprising the amino acid sequence SEQ ID NO:17 of the underlying application and identified as "peptide G".
- a porcine endogenous retrovirus (PoERV) ENV polypeptide fragment comprising the amino acid sequence SEQ ID NO:18 of the underlying application and identified as "peptide H".

a porcine endogenous retrovirus (PoERV) ENV polypeptide fragment comprising the amino acid sequence SEQ ID NO:19 of the underlying application and identified as "peptide J".

In view of the fact that antigenic and immunogenic porcine endogenous retrovirus (PoERV) polypeptide fragments are already known as well as their use for a serological assay to detect PoERV in a sample, considering the essential differences in amino acid sequence of the described solutions and due to the fact that no other technical features can be distinguished which, in the light of the prior art, could be regarded as special technical features, the IPEA agree with the ISA advice that there is no single inventive concept underlying the plurality of claimed inventions of the present underlying application in the sense of rule 131 PCT. Consequently, there is a lack of unity and the different inventions, not belonging to a common inventive concept, are considered as independent inventions.

**Re Item V**

**Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. The present application refers to a fragment of the GAG polypeptide of a porcine endogenous retrovirus (PoERV) comprising the amino acid sequence REERRDRRQEKNLTK and having a PoERV specific antigenic or immunogenic activity, to such a polypeptide comprising additionally a fragment of the PoERV ENV polypeptide, to an antiserum or an antibody specific to such a polypeptide fragment, and to the therapeutical or diagnostic use of said polypeptide fragments or antibodies.

**2. Reference is made to the following documents :**

D1: AKIYOSHI D E ET AL: 'IDENTIFICATION OF A FULL-LENGTH CDNA FOR AN ENDOGENOUS RETROVIRUS OF MINIATURE SWINE' JOURNAL OF VIROLOGY, THE AMERICAN SOCIETY FOR MICROBIOLOGY, US, vol. 72, no. 5, May 1998 (1998-05), pages 4503-4507.

D2: MATTHEWS A L ET AL: 'DEVELOPMENT AND VALIDATION OF A WESTERN IMMUNOBLOT ASSAY FOR DETECTION OF ANTIBODIES TO PORCINE ENDOGENOUS RETROVIRUS' TRANSPLANTATION, WILLIAMS AND WILKINS, BALTIMORE, MD, US, vol. 67, no. 7, 15 April 1999 (1999-04-15), pages 939-943, cited in the application

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**3. Lack of novelty; article 33(2) PCT.**

3.1 The document D2 discloses the development and the validation of a western immunoblot assay for the detection of antibodies to porcine endogenous retrovirus (Title). This test was developed for the diagnostic testing of porcine xenografts recipients (p. 939, left-hand column, lines 5-8). The polypeptide used for the test in D2 is the GAG polypeptide of the PoERV which comprises the amino acid sequence REERRDRRQEKNLTK. Moreover, said polypeptide has a PoERV specific antigenic or immunogenic activity.
The attention of the applicant is drawn to the fact that the authors of D2 were aware that the nucleotide sequence of a PoERV was already known (p. 940, left-hand column, lines 49-59).

Therefore, the subject-matter of claims 1-4, 13, 16, 18 and 19 can not be considered as novel in the sense of article 33(2) PCT.

3.2 The document D1 discloses the identification of a full-length cDNA of an endogenous retrovirus of miniature swine. According to the authors of D1, the existence of PoERV clones will enable studies of infection by

endogenous retroviruses in xenotransplantation (p. 4503, Abstract, lines 10-11). Table 1 of D1 (p. 4504) shows the identities between the GAG, POL and ENV polypeptides of PERV-MSL and the corresponding polypeptides of the porcine endogenous retroviruses tsukuba-1 and PK15-ERV.

Therefore, the subject-matter of claims 1-4 can not be considered as novel in the sense of article 33(2) PCT.

3.3 The document D3 discloses, inter alia, porcine retrovirus (PoEV) polypeptides, antibodies immuno-reactive with PoEV polypeptides, pharmaceutical compositions comprising recombinant PoEV polypeptides for use as prophylactic and/or therapeutic agents and the use of said polypeptides in the preparation of medicament for use in medicine (Abstract). D3 also claims the use of the recombinant polypeptide in the preparation of a vaccine (claim 27), the use of the polypeptide in therapy or diagnosis (claim 29) and the use of the polypeptide in the fabrication of a medicament (claim 30). The nucleotide and protein sequence of the GAG polypeptide of PoEV is given in Fig. 3 (nucleotides 588-2162). The GAG amino acid sequence disclosed in D3 is very similar to the GAG polypeptide of the present application and comprises the sequence REERRDRRQEKNLTK.

The IPEA considers that the GAG polypeptide of the PoEV disclosed in D3 has a PoERV specific antigenic or immunogenic activity.

Therefore, the subject-matter of claims 1-4, 13-14, 16, 18 and 19 can not be considered as novel in the sense of article 33(2) PCT.

**4. Lack of inventive step; article 33(3) PCT.**

The IPEA considers that the skilled person, knowing from D1 and D2 the nucleotide and corresponding amino acid sequences of porcine endogenous retrovirus (PoERV) and the fact that said PoERV may infect human cells, for example in the case of xenografts, would need no inventive activity to consider using the GAG polypeptide or antibodies directed against said polypeptide for the

therapy or diagnostic of an infection with PoERV, especially in the light of D2 which shows that the GAG polypeptide could be used for the detection of antibodies to porcine endogenous retrovirus.

Therefore, the subject-matter of claims 1-4 and 12-19 can not be considered as inventive in the sense of article 33(3) PCT.

The attention of the applicant is drawn to the fact that the selection of fragment comprising the amino acid sequence REERRDRRQEKNLTK could only be considered as inventive if motivated by a technical purpose, i.e. a hitherto unknown or unexpected effect due to the selection of said specific sequence. At the moment, the IPEA fails to see such a technical effect.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 February 2001 (22.02.2001)

PCT

(10) International Publication Number
WO 01/12816 A2

(51) International Patent Classification[7]: C12N 15/49, C07K 14/15, G01N 33/50, C07K 16/10, A61K 39/21

(21) International Application Number: PCT/GB00/03159

(22) International Filing Date: 16 August 2000 (16.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9919604.0 18 August 1999 (18.08.1999) GB

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published:**
— *Without international search report and to be republished upon receipt of that report.*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: RETROVIRUS ASSAY

## PoERV *GAG* POLYPEPTIDE SHOWING POSITIONS OF PEPTIDES

MGQTVTTPLSLTLDHWTEVRSRAHNLSVQVKKGPWQTFCASEWPTFDVGWPSEGTFNSEIILAVKAIIFQ
TGPGSHPDQEPYILTWQDLAEDPPPWVKPWLNKPRKPGPRILALGEKNKHSAEKVEPSSSYLPRDRGAAD
LAGTPTCSPTPLSSTGCCEGTSAPPGAPVVEGPAAGTRSRRGATPERTDEIAILPLRTYGPPMPGGQLQP
LQYWPFSSADLYNWKTNHPPFSEDPQRLTGLVESLMFSHQPTWDDCQQLLQTLFTTEERERILLEARKNV
PGADGRPTQLQNEIDMGFPLTRPGWDYNTAEGRESLKIYRQALVAGLRGASRRPTNLAKVREVMQGPNEP
PSVFLERLMEAFRRFTPFDPTSEAQKASVALAFIGQSALDIRKKLQRLEGLQEAELRDLVREAEKVYYRR
ETEEEKEQRKEKEREE**reerrdrrqeknltk**ILAAVVEGKSSRERERDFRKIRSGPRQSGNLGNRTPLDK
DQCAYCKEKGHW**arncpkkgnkgpkv**LALEEDKD

The *gag* peptides 1 and 2 are shown in lower case bold. Peptide 1: REERRDRRQEKNLTK; Peptide 2: ARNCPKKGNKGPKV.

(57) Abstract: The present invention relates to polypeptide fragments derived from porcine endogenous retrovirus (PoERV) GAG and ENV polypeptides, and to their use in detection of PoERV antibodies in a test sample. Also provided are antibodies to GAG and ENV polypeptides, which may be used to detect PoERV in a sample. Polypeptide sequences are provided which are common to several strains of PoERV, as are sequences specific to a single PoERV strain.

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# RETROVIRUS ASSAY

The present invention relates *inter alia* to porcine endogenous retrovirus (PoERV) fragments, in particular to *gag* and *env* fragments of PoERV. The invention relates further to use of such fragments in detection of PoERV or detection of exposure to PoERV. There is also provided antibodies to *gag* and *env* polynucleotides and polypeptides; kits for the detection of PoERV or exposure to PoERV; and *env* peptides and antisera specific for the various types of PoERV and use of such peptides and antisera in the detection of specific PoERV types.

Porcine endogenous retrovirus (PoERV) is an endogenous Gammaretrovirus present typically as a provirus found in several loci in the porcine genome. The proviral genome can be silent or is expressed. Expression of the virus was found to be associated with leukaemic pigs (Strandstrom et al, 1974) and some continuous porcine cell lines produce PoERV (Todaro et al, 1974). Virus from these cells has been shown to infect non-porcine cell-types including human cells (Galbraith et al, 1997; Patience et al, 1997). Three subgroups of PoERV have been described and are designated PoERV A, B and C dependent on the tropism of the virus and the related envelope gene structure (Onions et al, 1998). Only subtypes A and B have been shown to be capable of consistently infecting human cells in vitro. Subgroup C PoERV from mini-pigs has only been shown to infect one human cell line and this may reflect a low capacity for

infection of human cells. Since PoERV is expressed in pigs there is the potential for virus to be present in material prepared from pigs. Furthermore, as a consequence of xenotransplantation using porcine donor organs, there is the possibility that the endogenous virus will be expressed in vivo and be a potential risk of PoERV infection of the patient and the general population thereafter.

A number of different types of PoERV are known, based on their genetic makeup. Types designated PERV A, PERV B, and PoEV1 are described in International Patent Application WO97/40167, while types designated PERV MSL and Tsukuba are described in International Patent Application WO97/21836.

PoERV viruses comprise three genes: *gag*, *pol*, and *env*, generating GAG, POL and ENV polypeptides. It has been observed that the *gag* region of the genome appears to be substantially conserved among different viruses, as well as between PoERV virus types, while the *env* region contains both conserved and non-conserved regions, which non-conserved regions are observed to vary between viral types.

It is among the objects of the present invention to provide means whereby patients and/or samples may be monitored for viral infection. It is further among the objects of the present invention to provide means whereby the viral type may be determined.

According to one aspect of the present invention there is provided a PoERV polypeptide fragment, wherein said polypeptide fragment has PoERV specific antigenic or immunogenic activity. Antigenic or immunogenic activity is

to be understood as capable of eliciting a PoERV specific immune response when introduced into a normal mammalian host. For example, PoERV specific antibodies are produced as a consequence.

According to a further aspect of the present invention there is provided an antiserum specific to a PoERV polypeptide fragment as described above.

According to one aspect of the present invention, there is provided a fragment of a PoERV GAG polypeptide, wherein said fragment has PoERV specific antigenic or immunogenic activity. Such a fragment will be referred to hereinafter as a GAG peptide, it being understood that this is distinct from native GAG protein, and may comprise only a fragment thereof, provided the GAG peptide has antigenic activity. The consensus PoERV GAG polypeptide sequence is shown in Figure 1; in preferred embodiments of the invention, the GAG peptide may be selected from within this sequence.

According to a further aspect of the present invention, there is provided a fragment of a PoERV ENV polypeptide, wherein said fragment has PoERV specific antigenic or immunogenic activity. Such a fragment will be referred to hereinafter as an ENV peptide, it being understood that this is distinct from native ENV protein, and may comprise only a fragment thereof, provided the ENV peptide has antigenic activity. In one embodiment, the ENV peptide may be selected from within a conserved region of the various PoERV sequences, as illustrated in Figures 2

and 3. Conserved regions are those which comprise identical and/or highly conserved amino acid sequences in different PoERV virus types; wholly conserved amino acids are indicated in Figures 2 and 3 by an asterisk beneath the amino acid, with highly conserved amino acids being indicated by a colon. Alternatively, the ENV peptide may be selected from within the non-conserved regions of the various PoERV sequences of Figures 2 and 3, in which case the ENV peptide will be specific for a particular type of PoERV. Specific examples of non-conserved type-specific ENV peptides are shown as peptides D-H and J in Figure 3 In a third embodiment, the ENV peptide may comprise both a conserved and a non-conserved region of the PoERV ENV protein, from either adjacent or non-adjacent regions of the ENV protein. Such peptides may be considered useful in simultaneous detection of any PoERV virus and a specific viral type.

In a further aspect of the present invention, there is provided a fusion GAG/ENV peptide, which peptide comprises both GAG peptide sequences and ENV peptide sequences. Such peptide may be considered useful in simultaneous detection of any PoERV virus, by means of the GAG peptide, and a specific viral type, by means of the ENV peptide.

According to a yet further aspect of the present invention there is provided antibodies specific to either GAG or ENV peptides. The antibodies may be polyclonal or monoclonal. Such antibodies can include, but are not limited to, polyclonal antibodies, monoclonal antibodies

(mAbs), humanized or chimeric antibodies, single chain antibodies, Fab fragments, $F(ab')_2$ fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. The Ig tails of such antibodies can be modified to reduce complement activation and Fc binding, (See, for example, European Patent No. 239400 B1, Aug. 3, 1994).

For the production of antibodies to a peptide, various host animals can be immunized by injection with a peptide, or a portion thereof. Such host animals can include but are not limited to rabbits, mice, and rats, to name but a few. Various adjuvants can be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Gurein) and *Corynebacterium parvum*.

Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen, such as target gene product, or an antigenic functional derivative thereof. For the production of polyclonal antibodies, host animals such as those described above, can be immunized by injection with a gene product supplemented with adjuvants as also described above.

Monoclonal antibodies, which are homogeneous populations of antibodies to a particular antigen, can be obtained by any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to the hybridoma technique of Kohler and Milsrein, (1975, Nature 256:495-497; and US Pat. No. 4,376,110), the human B-cell hybridoma technique (Kosbor et al., 1983, Immunology Today 4:72; Cole et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:2026-2030), and the EBV-hybridoma technique (Cole et al., 1985, Monoclonal Anti-bodies And Cancer Therapy, Alan R. Liss, Inc., pp.77-96).

Such antibodies can be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAb of this invention can be cultivated in vitro or in vivo. Production of high titers of mAbs in vivo makes this the presently preferred method of production.

In addition, techniques for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger et al., 1984, Nature, 312:604-608; Takeda et al., 1985, Nature, 314:452-454; U.S. Pat. No. 4,816,567) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable

region derived from a murine mAb and a human immunoglobulin constant region.

Alternatively, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778: Bird, 1988, Science 242:423-426; Huston et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:5879-5883; and Ward et al., 1989, Nature 334:544-546) and for making humanized monoclonal antibodies (U.S. Pat. No. 5,225,539) can be utilized to produce anti-differentially expressed or anti-pathway gene product antibodies.

Antibody fragments which recognize specific epitopes can be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragments. Alternatively, Fab expression libraries can be constructed (Huse et al., 1989, Science, 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

Further aspects of the present invention provide methods of screening serum or tissue from humans or animal recipients of porcine tissue for exposure to PoERV. These methods include: use of antibodies to GAG or ENV peptides in the capture and/or detection of PoERV antigens; use of antibodies to GAG or ENV peptides in the detection of PoERV gene expression in virus infected cells by indirect immunofluorescence staining; the use of antibodies to GAG

or ENV peptides in the visualisation of PoERV virions in a sample by immuno-electron microscopy; use of GAG or ENV peptides in Western blotting for the detection of PoERV antibodies in samples from recipients of porcine-derived materials; and the use of GAG or ENV peptides in an enzyme-linked immunosorbent assay (ELISA) for the detection of PoERV antibodies.

Each of these methods may non-specifically detect any PoERV type (if GAG peptides, conserved ENV peptides or antibodies are used) or specific PoERV types, if type-specific non-conserved ENV peptides or antibodies are used.

The present invention also encompasses assay kits including GAG or ENV peptides or antibodies to such peptides, for use in the abovementioned assays. In preferred embodiments, the kits may further comprise any or all necessary preparative reagents, washing reagents, detection reagents and signal producing reagents commonly known in the art.

In all of these assays and methods, a number of distinct peptides or antibodies may be used, either sequentially or simultaneously, and differently labelled, in order to detect a number of different PoERV types in a single assay.

Diagnostic assays based upon the present invention may be used to determine the presence or absence of PoERV infection, and the PoERV type involved.

In an assay for the diagnosis of viral infection, there are basically three distinct approaches that can be adopted, involving the detection of viral nucleic acid, viral antigen or viral antibody respectively. Viral nucleic acid is generally regarded as the best indicator of the presence of the virus itself and would identify materials likely to be infectious. However, the detection of nucleic acid is not usually as straightforward as the detection of antigens or antibodies since the level of target can be very low. Viral antigen is used as a marker for the presence of virus and as an indicator of infectivity. Depending upon the virus, the amount of antigen present in a sample can be very low and difficult to detect. Antibody detection is relatively straightforward because, in effect, the host immune system is amplifying the response to an infection by producing large amounts of circulating antibody. The nature of the antibody response can often be clinically useful, for example IgM rather than IgG class antibodies are indicative of a recent infection, or the response to a particular viral antigen may be associated with clearance of the virus. Thus the exact approach adopted for the diagnosis of a viral infection depends upon the particular circumstances and the information sought. In the case of PoERV, a diagnostic assay may embody any one or a combination of these three approaches.

In an assay for the diagnosis of PoERV involving detection of viral antigen or antibody, the method may comprise contacting a test sample with a peptide of the present invention or a polyclonal or monoclonal antibody against the peptide and determining whether there is any antigen-antibody binding contained within the test sample. For this purpose, a test kit may be provided comprising a peptide, as defined herein, or a polyclonal or monoclonal antibody thereto and means for determining whether there is any binding with antibody or antigen respectively contained in the test sample to produce an immune complex. The test sample may be taken from any appropriate tissue or physiological fluid, such as blood (e.g. serum or plasma), saliva, urine, cerebrospinal fluid, sweat, tears or tissue exudate. If a physiological fluid is obtained, it may optionally be concentrated for any viral antigen or antibody present.

A variety of assay formats may be employed. The peptide can be used to capture selectively antibody against PoERV from solution, to label selectively the antibody already captured, or both to capture and label the antibody. In addition, the peptide may be used in a variety of homogeneous assay formats in which the antibody reactive with the peptide is detected in solution with no separation of phases.

The types of assay in which the peptide is used to capture antibody from solution involve immobilization of the peptide on to a solid surface. This surface should be

capable of being washed in some way. Examples of suitable surfaces include polymers of various types (moulded into microtitre wells; beads; dipsticks of various types; aspiration tips; electrodes; and optical devices), particles (for example latex; stabilized red blood cells; bacterial or fungal cells; spores; gold or other metallic or metal-containing sols; and proteinaceous colloids) with the usual size of the particle being from 0.02 to 5 microns, membranes (for example of nitrocellulose; paper; cellulose acetate; and high porosity/high surface area membranes of an organic or inorganic material).

The attachment of the peptide to the surface can be by passive adsorption from a solution of optimum composition which may include surfactants, solvents, salts and/or chaotropes; or by active chemical bonding. Active bonding may be through a variety of reactive or activatable functional groups which may be exposed on the surface (for example condensing agents; active acid esters, halides and anhydrides; amino, hydroxyl, or carboxyl groups; sulphydryl groups; carbonyl groups; diazo groups; or unsaturated groups). Optionally, the active bonding may be through a protein (itself attached to the surface passively or through active bonding), such as albumin or casein, to which the viral peptide may be chemically bonded by any of a variety of methods. The use of a protein in this way may confer advantages because of isoelectric point, charge, hydrophilicity or other physico-chemical property. The viral peptide may also be attached to the surface (usually

but not necessarily a membrane) following electrophoretic separation of a reaction mixture, such as immunoprecipitation.

After contacting the surface bearing the peptide with a test sample (in the presence of a blocking mixture if required), allowing time for reaction, and, where necessary, removing the excess of the sample by any of a variety of means, (such as washing, centrifugation, filtration, magnetism or capillary action) the captured antibody is detected by any means which will give a detectable signal. For example, this may be achieved by use of a labelled molecule or particle as described above which will react with the captured antibody or any molecule containing an epitope contained in the peptide. In one embodiment, it is preferred to add an anti-human IgG conjugated to horseradish peroxidase and then to detect the bound enzyme by reaction with a substrate to generate a colour.

The detectable signal may be produced by any means known in the art such as optical or radioactive or physico-chemical and may be provided directly by labelling the molecule or particle with, for example, a dye, radiolabel, fluorescent, luminescent, chemiluminescent, electroactive species, magnetically resonant species or fluorophore, or indirectly by labelling the molecule or particle with an enzyme itself capable of giving rise to a measurable change of any sort. Alternatively the detectable signal may be obtained using, for example, agglutination, or through a

diffraction or birefrigent effect if the surface is in the form of particles.

Assays in which a peptide itself is used to label an already captured antibody require some form of labelling of the peptide which will allow it to be detected. The labelling may be direct by chemically or passively attaching for example a radiolabel, magnetic resonant species, particle or enzyme label to the peptide; or indirect by attaching any form of label to a molecule which will itself react with the peptide. The chemistry of bonding a label to the peptide can be directly through a moiety already present in the peptide, such as an amino group, or through an intermediate moiety, such as a maleimide group. Capture of the antibody may be on any of the surfaces already mentioned in any reagent including passive or activated adsorption which will result in specific antibody or immune complexes being bound. In particular, capture of the antibody could be by anti-species or anti-immunoglobulin-sub-type, by rheumatoid factor, proteins A, G and the like, or by any molecule containing an epitope contained in the peptide.

The labelled peptide may be used in a competitive binding fashion in which its binding to any specific molecule on any of the surfaces exemplified above is blocked by antigen in the sample. Alternatively, it may be used in a non-competitive fashion in which antigen in the sample is bound specifically or non-specifically to any of the surfaces above and is also bound to a specific bi- or

poly-valent molecule (e.g. an antibody) with the remaining valencies being used to capture the labelled peptide.

Examples of the invention will now be described by way of illustration only, and with reference to the accompanying Figures, in which:

Figure 1 is the consensus amino acid sequence of the PoERV GAG protein;

Figure 2 is a comparison of amino acid sequences of five different PoERV ENV proteins; and

Figure 3 is a comparison of amino acid sequences of the variable region of five different PoERV ENV proteins, showing the six different ENV peptides (peptides D-H and J) referred to in the following examples.

**METHODS**

**Preparation of PoERV virions.**

Human 293 cells (American Type Culture Collection [ATCC] # CRL1573) and Raji cells (ATCC # CCL 86 ) were infected with PoERV by exposure to polybrene (Sigma-Aldrich Co. Ltd.) and continued incubation with cell-free filtered supernatant from PK-15 (ATCC # CCL 33) cells previously shown to be infected with all three subgroups of PoERV. The 293 cells allow replication of type B PoERV (POEV-1). The 293 cells were shown to be infected after passage by measurement of the reverse transcriptase activity of the cell supernatant and by a PoERV GAG-specific Polymerase Chain Reaction (PCR). The resulting virus particles were isolated from the cell line supernatant as follows.

Supernatant from exponentially growing cells was layered onto a 20/40% (w/v) discontinuous sucrose density gradient and centrifuged at 100,000 g for 150 min. The viral material at the sucrose interface was harvested, and viral particles pelleted by further ultracentrifugation at 100,000g for 60 min, followed by resuspension in DMEM (Life Technologies Ltd., UK).

**Control retroviruses**

To provide retroviral controls for cross reactivity with PoERV GAG and ENV, Squirrel monkey retrovirus, Murine leukaemia virus, Maedi-Visna virus and Equine infectious anemia virus virions were prepared from the appropriate infected cell line as described by Shepherd and Smith (1999).

**Selection and preparation of GAG peptides**

Peptides from the GAG protein can provide a capture antigen and a means to generate positive control antisera. The antisera can be directed against conserved polypeptides present in the PoERV virion core likely to induce an immune response in recipients of the virus. These reagents would be useful diagnostic tools for immunosurveillance of recipients of porcine material or tissues for exposure to PoERV. Therefore, peptides encompassing potential antigenic regions of PoERV GAG were selected from the translated amino-acids derived from the sequence of the gag region of PoERV based on three criteria; hydrophilicity,

potential ß-turns and K, D, R and E charged residues. The regions were identified using Hopp and Woods hydropathicity (1981) scale and Kyte and Doolittle (1982) hydrophobicity scale.

For GAG two peptides were identified as potential antigens. Peptide 1 was from the C-terminus of p30-GAG at residue 437-451 of the polypeptide, nucleotides 1896-1940 of the *gag* open reading frame (ORF). Peptide 2 was from the start of the p10 segment of the GAG polypeptide at residue 502-515 of the polypeptide, nucleotides 2091- 2132 of the *gag* ORF. The peptides shown below were chemically synthesised by Genosys Biotechnologies Inc.

Peptide 1: (C)REERRDRRQEKNLTK

Peptide 2: (W)ARNCPKKGNKGPKS

The bracketed amino-acid is not in sequence - 5' position is from next residue (R).

These peptides, and the GAG consensus polypeptide sequence, are shown in Figure 1.

A BLAST search (Altschui et al., 1997) of the non-redundant GenBank coding sequences with GAG peptide 1 showed homology with seven sequences all from the *gag* ORF. Of the seven, three were with PoERV sequences with accessions gi 3116446 (100% match), emb CAA7651 (100% match), gi 3116442 (86% match). The remainder were against the closely related Gibbon ape leukaemia virus (gi 3033415, 92% match) and Simian sarcoma virus (sp PO3330, 86% match).

The remaining two sequences were from murine viruses, including a virus from *Rattus norvegicus* (emb CAA24514; 92% match), and one against *Mus dunni* endogenous retrovirus (gi 3309124, 93% match). A similar BLAST search with GAG peptide 2 showed 100 - 99% homology with only the PoERV sequences listed above.

**Selection and Preparation of PoERV ENV peptides**

We have previously identified and derived the nucleotide sequence of a unique PoERV type capable of infecting human cells (Galbraith, 1997). Furthermore, it has been shown that the amino acid sequence of the ENV region of various PoERV types contains both conserved and non-conserved regions (Galbraith, 1997; Figure 2). In order to exploit these differences to produce immunological reagents to allow the identification of the type of PoERV giving rise to an immunological reaction in a patient, PoERV-type-specific ENV peptides and antisera were generated.

Six peptides, D-H and J, were identified as potential antigens. The peptides are shown below and their position in the *env* ORF of the various PoERV types is shown in Figure 3. The peptides were chemically synthesised by the University of Glasgow Veterinary Pathology Department.

Peptide D: TSLRPDITQPPSNSTT

Peptide E: KGKQENIQKWINGMS

Peptide F: RKTGKYSKVDKWYELGNS

Peptide G: NTVLTGQRPPTQ

Peptide H: GHGRWKDWQQRVQKDVRNKQIS

Peptide J: IQEQRPSPNPSDYNTT

The amino acid residues of all peptides are identified by the standard one letter abbreviations.

**Preparation of recombinant PoERV p30-GAG and ENV polypeptides**

In addition to the peptide reagents more general PoERV p30-GAG and an abbreviated ENV polypeptides were designed and produced for use as capture antigens and to produce anti-polypeptide sera. The required polypeptide portions of the *gag* and *env* genes were produced by PCR amplification, molecularly cloned into a prokaryotic expression vector and expressed as described below using standard techniques (Maniatis et al, 1982).

**PoERV p30-GAG**

A fragment encompassing the p30 region of the *gag* ORF from nucleotide 1173-1949 of the PoERV genome (Galbraith et al, 1997; Gene Bank Accession # A66553) was amplified by PCR from cDNA generated from PK15 mRNA using ligation independent cloning oligonucleotide primers (pET-32 Ek/LIC cloning and expression vector; Novagen Inc. Catalogue # 69076-3). The oligonucleotides were:

p30 forward 5' GAC GAC GAC AAG **CTG CGC ACC TAT GGC C** 3'

p30 reverse 5' GAG GAG AAG CCC GGG **TCT AGG CCA AGA TCT TAG TCA AAT TCT TCT C** 3'

The nucleotides in bold are viral specific.

The PCR conditions were 30 cycles of 95°C for 1 min, 58°C for 1 min and 72°C for 1 min. The resulting 776 base pair fragment was molecularly cloned into the appropriate LIC site of the pET-32 LIC vector following the manufacturer's instructions (Novagen Inc. 69076-3 instruction manual), transfected into competent Novoblue™ *Escherichia coli* cells and plated on solid LB medium containing ampicillin. The transformed colonies were selected by resistance to ampicillin.

**PoERV ENV**

A fragment encompassing the region of the *env* ORF from nucleotide 5616- 6304 of the PoERV genome (Galbraith et al,1997; Gene Bank Accession # A66553) was amplified by PCR from cDNA generated from PK15 and PoERV-infected 293 cells (PoERV B) mRNA using ligation independent cloning oligonucleotide primers (pET-32 Ek/LIC cloning and expression vector; Novagen Inc. Catalogue # 69076-3). The oligonucleotides were:

*env* forward 5' GAC GAC GAC AAG **ATC CAT GCA TCC CAC GTT** 3'

*env* reverse 5' GAG GAG AAG CCC GGT **CTC TAT CCT AAG GCG** 3'

The nucleotides in bold are viral specific.

The PCR conditions were 30 cycles of 95°C for 1 min, 60°C for 1 min and 72°C for 1 min. The resulting 688 base pair fragment was molecularly cloned into the appropriate LIC site of the pET-32 LIC vector following the manufacturer's instructions as described above.

For expression from the T7 promoter the recombinants are required to be transferred to a host with T7 polymerase activity. To this end plasmid DNA was isolated from the ampicillin resistant Novoblue™ clones carrying the *gag* or *env* fragment in the correct orientation for expression as determined by restriction endonuclease mapping. The plasmid DNAs were each transfected into competent *E. coli* AD494 (DE3) *trx B-* .

For screening for the production of recombinant protein, two ml cultures of *E. coli* transformed with either of the two expression constructs were grown with shaking at 37°C to late log phase ($O.D._{600nm}$ of approximately 0.6) and induced by the addition of Isopropylthio-beta-galactoside (IPTG) to 0.1 mM. Induced cultures were then incubated for a further 2 h after which the bacteria were collected by centrifugation. The bacterial pellet was lysed by boiling in SDS-PAGE sample buffer and the protein profile of the induced bacteria was analysed on a 12% acrylamide gel

followed by staining with coomasie brilliant blue dye. (Laemmli, 1970; Gallagher, 1997).

Large scale preparations of purified GAG and ENV polypeptides were made according to the manufacturer's instructions (Novagen Inc. Catalogue # 69076-3).

**Preparation of antisera to whole virions, peptides and recombinant P30-GAG and ENV antigens.**

For GAG peptide 1 and peptide 2, the peptides were conjugated with keyhole limpet hemacyanin carrier protein and each of two rabbits was inoculated six times at fourteen day intervals. The animals were bled out at day seventy seven after the first inoculation. The p30-GAG polypeptide was inoculated three times at fourteen day intervals into a rabbit. The animal was bled out at day seventy seven.

For ENV peptides D-H and J, the peptides were conjugated with keyhole limpet hemacyanin carrier protein and one sheep was inoculated three times at twenty eight day intervals. The ENV polypeptide was inoculated three times at fourteen day intervals into a rabbit.

Virions purified from PK15 cells were inoculated three times at fourteen day intervals into each of two guinea pigs.

**Indirect immunofluor scence staining**

To test the specificity of the p30-GAG antisera the method outlined by Riggs (1989) was used. PoERV-infected Raji cells and uninfected control Raji cells were fixed and tested for indirect immunofluorescence with anti p30-GAG using a fluorescein isothiocyanate (FITC) labelled anti-rabbit detector antibody. The cells were examined by fluorescence microscopy.

**Preparation of Western blot membranes**

Recombinant p30-GAG polypeptide and ENV polypeptide were prepared, harvested and purified from an *E.coli* vector. The recombinant proteins were tested to determine an appropriate dilution of protein which yielded a positive result in the immunoassay. In addition, extracts from PoERV-infected 293 cells, PoERV-infected Raji cells or purified PoERV virions were used as antigens. To obtain specific and reproducible Western blot assays, a number of parameters were required to be optimised for each assay, such as: Primary antibody dilution, incubation time, incubation temperature, secondary antibody dilution, incubation time, incubation temperature, washing buffers, blocking/dilution buffers, developing reagents. Recombinant polypeptides were added to nine wells of a ten lane 12% Tris/glycine acrylamide gel. Molecular weight markers were added to the first lane. The samples were electrophoresed and the gel electroblotted to a poly vinylidene fluoride (PVDF) membrane. (Gallagher et al,

1997). The membrane was cut into strips each strip containing one lane of recombinant protein. These strips were used as the basis of the assay.

**Preparation dilutions of antisera**

Samples were prepared in a Class 2 safety cabinet or other clean environments.

A typical negative control was prepared by making up to a 1:200 dilution of normal sera in blocking reagent.

A typical positive control was prepared by making a 1: 500, 1: 1000 or greater dilution of anti-PoERV p30-GAG polypeptide, peptide serum or anti - recombinant ENV serum.

A typical test serum was prepared by making up to a 1:200 dilution of sera.

**Preparation of Western blotting membranes and PoERV antibody detection.**

To block non-specific binding sites membrane strips each were placed in a 15 ml centrifuge tube and 2 ml blocking reagent (2.5 g skimmed dried milk in 50 ml PBS/ 0.5% v/v Tween-20™) added. The strips were placed on a rotary shaker such that the strip moved slightly on each revolution and were incubated for 30 min at ambient

temperature. The blocking reagent was removed and replaced with 5-10 µl of the diluted serum. The membrane was incubated with shaking for 1 h at ambient temperature. To stop incubation the strip was removed from diluted serum and placed into PBS/ Tween-20™ and washed with three changes of PBS/ Tween-20™ at ambient temperature with shaking.

The appropriate species specific secondary antiserum conjugated to alkaline phosphatase was used as detector e.g. if human serum was being tested, an anti-human IgG alkaline phosphatase (AP) conjugate was used. The p30-GAG positive control required anti-rabbit IgG AP conjugate for detection and the anti ENV required anti-sheep IgG AP conjugate. The detection was done as follows; each strip was placed in an unused 15 ml centrifuge tube, 2 ml of 1:1000 dilution of secondary sera in blocking reagent was added and incubated with shaking at ambient temperature for 1 h. The strip was removed from the centrifuge tube, placed in PBS/Tween-20™ and washed with 3 changes of PBS/Tween-20™, at ambient temperature with shaking. The strips were then put into a 15 ml centrifuge tube and 2 ml of bromochlorindoyl phosphate/ nitroblue tetrazolium (BCIP/NBT, Sigma-Aldrich Co. Ltd.) solution was added to each tube. The strips were shaken gently and allowed to develop for 5 min. The reaction was stopped by rinsing the membrane strip in purified water and the strips were removed from the water and allowed to air dry.

**ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)**

**Antigen coating of microtiter plat s**

50 µl of antigen (either recombinant p30-GAG, extracts from PoERV-infected 293 cells or purified PoERV B virions) were diluted to the required concentration in carbonate-bicarbonate coating buffer (Sigma-Aldrich Co. Ltd.) was added to wells of a 96 well, flat bottomed microtiter ELISA plate (Dynex Immulon 2). Some wells contained carbonate-bicarbonate coating buffer only and some were left blank to act as controls for non-specific binding. The plate was covered with a plate seal and incubated at 4°C for approximately 16 h. The unbound antigen and coating solution were then removed from the wells with a pipette and washed three times with PBS / 0.05% Tween-20™. Any remaining PBS / 0.05% Tween-20™ was removed by blotting on a tissue.

**Blocking of microtiter plates**

50 µl of fresh blocking buffer (5% (w/v) skimmed milk /PBS / 0.05% Tween-20™) was added to each antigen coated well and control well. The plate was covered with a plate seal and incubated at ambient temperature in an humidified chamber for 1 h. The blocking buffer was removed and the plate wells washed three times with PBS / 0.05% Tween-20™ and any remaining PBS / 0.05% Tween-20™ was removed by blotting on a tissue.

**Incubation with primary antibody**

50 µl of negative control sera, test sera, and positive control sera at the experimental dilution were added to the antigen coated wells and the plates covered with a plate seal and incubated at ambient temperature for approximately 1 h in an humidified chamber. Following incubation all sera were removed using a pipette and the plate wells washed three times with PBS / 0.05% Tween-20™, any remaining PBS / 0.05% Tween-20™ was removed by blotting on a tissue.

**Incubation and development with peroxidase-conjugated secondary antibody**

50 µl of a 1:500 dilution of species specific peroxidase conjugated secondary antibody in blocking buffer was added to each antigen coated well. For human serum an anti-human IgG peroxidase conjugate was used. The p30-GAG positive control required anti-rabbit IgG peroxidase conjugate for detection and the anti ENV required anti-sheep IgG peroxidase conjugate. The plates were covered with a plate seal and incubated at ambient temperature for approximately 1 h in an humidified chamber. Following incubation the conjugate was removed using a pipette and the plate wells washed three times with PBS / 0.05% Tween-20™. Any remaining PBS / 0.05% Tween-20™ was removed by blotting on a tissue.

The substrate was prepared as follows: one O-phenylenediamine tablet (Sigma-Aldrich Co. Ltd.) and one urea/$H_2O_2$ tablet were dissolved in 20 ml of purified water.

An aliquot of 50 µl of substrate was added to each w ll and the plate incubated at ambient temperature in the dark for 30 min. The reaction was then stopped by adding 50 µl of 3N HCl or 3 M $H_2SO_4$ to each well. The colour development in the wells was measured at 490 nm using a Dynex MRX microplate reader.

For alkaline phosphatase conjugated secondary antibodies the substrate used was p-Nitrophenyl phosphate (pNPP; Sigma-Aldrich Co. Ltd.) and the plates were read at 405 nm.

**Electron Microscopy**

Negative stain electron microscopy (NSEM) (Doane, 1980) was used to identify the presence of PoERV virions. Supernatant from PoERV-infected pK15 cells was layered onto a 20/40% (w/v) discontinuous sucrose density gradient and centrifuged at 100,000 g for 150 min. The viral material at the sucrose interface was harvested, and viral particles pelleted by further ultracentrifugation at 100,000g for 60 min, followed by resuspension in DMEM (Life Technologies Ltd.). The sample was then applied to pioloform-coated copper 300 mesh EM grids and allowed to air dry. Grids were fixed with 2.5% glutaraldehyde (Agar Scientific), stained with 5% uranyl acetate (Agar Scientific) and allowed to air dry. Grids were examined on a Philips EM-400 transmission electron microscope.

**Immuno- Electron Microscopy**

The immunostaining was done following the method of Marshall et al (1992). Briefly, NSEM samples were applied to pioloform-coated nickel 300 mesh EM grids and allowed to air dry. Grids were fixed with modified immunofix, post fixed with 0.5 M $NH_4Cl$, then incubated with 2% bovine serum albumin (Sigma-Aldrich Co. Ltd.). Samples were then incubated with rabbit anti-PoERV (rabbits immunised with whole PoERV) or rabbit anti-PoERV p30-GAG antibody, washed in modified immunobuffer followed by incubation with anti-rabbit IgG gold conjugate (Sigma-Aldrich Co. Ltd.). Grids were stained with 5% uranyl acetate, and allowed to air dry. Samples were visualised on a Philips EM-400 transmission electron microscope.

**EXAMPLE ONE**

**Indirect immunofluorescence staining of PoERV-infected cells.**

Viral specific fluorescence was observed in PoERV-infected Raji cells using the anti p30-GAG antiserum. No immunofluorescence was seen with the negative control Raji cells.

**EXAMPLE TWO**

**Western Blotting for Antibodies to PoERV GAG**

Anti-GAG peptide 1 antisera and sera from rabbits inoculated with the recombinant p30-GAG polypeptide detected the expected protein of approximately 30kd in extracts of PoERV-infected 293 or PoERV-infected Raji cells, purified PoERV virions and recombinant p30-GAG. The PoERV antibody could be detected at a dilution of 1:1000.

No band of equivalent size to the GAG 30 kd polypeptide was detected in uninfected control cells.

No band of equivalent size to the GAG 30 kd polypeptide was detected against the following purified retroviruses:

Squirrel monkey retrovirus
Murine leukaemia virus
Maedi-Visna virus
Equine infectious anemia virus

Therefore, the positive control antisera were specific for PoERV.

**Determination of assay specificity using Serum Panels**

**Normal human sera**

On testing of 90 normal serum samples from healthy individuals, whose blood was taken for occupational health

reasons, no PoERV reactive antibody was detected in any of the sera.

**Normal primate sera**

On testing 42 normal serum samples from healthy primates no PoERV reactive antibody was detected. There was no cross-reactivity with sera from normal primates.

**Cardiac transplant patient sera**

On testing 20 serum samples from individuals who had received a cardiac transplant in the preceding 36 months no PoERV reactive antibody was detected. There was no cross-reactivity with sera from patients who had been immunosupressed.

**HIV positive sera**

On testing 13 serum samples from individuals positive for the presence of antibody to HIV no PoERV reactive antibody was detected. There was no cross-reactivity with sera from patients infected with a human Lentivirus.

**HTLV positive sera**

On testing 10 serum samples from individuals positive for the presence of antibody to HTLV-1 virus no PoERV reactive antibody was detected. There was no cross-reactivity with sera from patients infected with a human Gammaretrovirus.

**Butch rs with acute lymphoblastic leukaemia (ALL)**

On testing 3 serum samples from butchers with ALL no PoERV reactive antibody was detected. There was no cross-reactivity with sera from patients with assumed prolonged exposure to PoERV and PoERV antigens with a leukaemic disorder.

**EXAMPLE THREE**

**ELISA p30-GAG**

A titration of p30-GAG antigen to anti-p30-GAG antisera gave a significant signal at 1:250600 dilution antigen to 1:32000 dilution of antisera. A similar titration of antisera against PoERV virions gave a significant signal at a 1:3200 dilution of both antigen and antisera.

**Normal human sera**

On testing of five normal serum samples from healthy individuals whose blood was taken for occupational health reasons, no significant signal was detected in any of the sera against recombinant p30-GAG.

**EXAMPLE FOUR**

**Detection and Visualisation of PoERV Virions by Immuno-Electron Microscopy**

Examination of PoERV virion preparations by negative stain revealed particles showing the characteristic size and structure of a Gammaretrovirus of approximately 90-120 nm with a dark inner core and double membraneous outer region.

The particles bound immuno-gold labeled anti p30-GAG antiserum indicating that the antiserum could be used to visualise PoERV virions by immuno-electron microscopy.

**EXAMPLE FIVE**

**ELISA ENV**

A titration of antisera raised against ENV peptides D, E, F, G, H, J. to purified PoERV B virions gave a significant signal indicating that the ENV peptides produced a virus-specific reaction in the animals. Peptides D and F, both from PoERV B (POEV1; Figure 2; Galbraith et al, 1997) gave the highest signal.

**EXAMPLE SIX**

**Western Blotting for Antibodies to PoERV ENV**

Antisera from guinea pigs inoculated with whole purified PoERV virions from PK15 cells detected the expected recombinant ENV protein of approximately 24 kD in extracts of *E.coli* expressing the *env* construct.

No band of equivalent size to the ENV 24 kD polypeptide was detected on *E.coli* control cells without the expresion construct.

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## CLAIMS

1. A porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment has PoERV specific antigenic or immunogenic activity.

2. A polypeptide fragment according to claim 1, wherein said polypeptide fragment comprises a fragment of a PoERV GAG polypeptide.

3. A polypeptide fragment according to claim 2, wherein said polypeptide fragment comprises a fragment of a polypeptide sequence as shown in Figure 1.

4. A polypeptide fragment according to claim 3, wherein said polypeptide fragment comprises the amino acid sequence REERRDRRQEKNLTK.

5. A polypeptide fragment according to claim 3, wherein said polypeptide fragment comprises the amino acid sequence ARNCPKKGNKGPKV.

6. A polypeptide fragment according to claim 1, wherein said polypeptide fragment comprises a fragment of a PoERV ENV polypeptide.

7. A polypeptide fragment according to claim 6 wherein said polypeptide fragment comprises a fragment of a polypeptide sequence as shown in Figures 2 or 3.

8. A polypeptide fragment according to claim 7 wherein said polypeptide fragment comprises a fragment from within a conserved region of the sequences shown in Figures 2 and 3.

9. A polypeptide fragment according to claim 7 wherein said polypeptide fragment comprises a fragment from within a non-conserved region of the sequences shown in Figures 2 and 3.

10. A polypeptide fragment according to claim 9 wherein said polypeptide fragment comprises an amino acid sequence selected from the sequences of peptides D, E, F, G, H and J as shown in Figure 3.

11. A polypeptide fragment according to claim 7 wherein said polypeptide fragment comprises a fragment from within a conserved region of the sequences shown in Figures 2 and 3, and a fragment from within a non-conserved region of the sequences shown in Figures 2 and 3.

12. A polypeptide fragment according to claim 1, wherein said polypeptide fragment comprises a fragment of a PoERV GAG polypeptide and a fragment of a PoERV ENV polypeptide.

13. An antiserum specific to a polypeptide fragment in accordance with any preceding claim.

14. A PoERV specific antibody or fragment thereof raised against a polypeptide fragment in accordance with any of claims 1 to 12.

15. Use of an antibody according to claim 14 in the detection of PoERV in a sample.

16. Use of a polypeptide fragment according to any one of claims 1 to 12 in the detection of PoERV antibodies in a sample.

17. An assay kit for use in detection of PoERV in a sample, the kit comprising an antibody in accordance with claim 14.

18. An assay kit for use in detection of PoERV antibodies in a sample, the kit comprising a polypeptide fragment in accordance with any of claims 1 to 12.

19. Use of an antibody according to claim 14, or a polypeptide fragment according to any one of claims 1 to 12 in therapy or diagnosis.

# FIGURE 1

## PoERV *GAG* POLYPEPTIDE SHOWING POSITIONS OF PEPTIDES

MGQTVTTPLSLTLDHWTEVRSRAHNLSVQVKKGPWQTFCASEWPTFDVGWPSEGTFNSEIILAVKAIIFQ
TGPGSHPDQEPYILTWQDLAEDPPPWVKPWLNKPRKPGPRILALGEKNKHSAEKVEPSSSYLPRDRGAAD
LAGTPTCSPTPLSSTGCCEGTSAPPGAPVVEGPAAGTRSRRGATPERTDEIAILPLRTYGPPMPGGQLQP
LQYWPFSSADLYNWKTNHPPFSEDPQRLTGLVESLMFSHQPTWDDCQQLLQTLFTTEERERILLEARKNV
PGADGRPTQLQNEIDMGFPLTRPGWDYNTAEGRESLKIYRQALVAGLRGASRRPTNLAKVREVMQGPNEP
PSVFLERLMEAFRRFTPFDPTSEAQKASVALAFIGQSALDIRKKLQRLEGLQEAELRDLVREAEKVYYRR
ETEEEKEQRKEKEREE**reerrdrrqeknltk**ILAAVVEGKSSRERERDFRKIRSGPRQSGNLGNRTPLDK
DQCAYCKEKGHW**arncpkkgnkgpkv**LALEEDKD

**Figure 1**. The *gag* peptides 1 and 2 are shown in lower case bold. Peptide 1: REERRDRRQEKNLTK; Peptide 2: ARNCPKKGNKGPKV.

## FIGURE 2

## ALIGNMENT OF DIFFERENT PoERV ENVELOPE PROTEINS

```
PERVA      MHPTLSRRHLPIRGGKPKRLKIPLSFASIAWFLTLSITPQVNGKRLVDSPNSHKPLSLTW
POEVMSL    MHPTLNRRHLPIRGGKPKRLKIPLSFASIAWFLTLSITSQTNGMRIGDSLNSHKPLSLTW
TSUKUBA    MHPTLSRRHLPIRGGKPKRLKIPLSFASIAWFLTLSITSQTNGMRIGDSLNSHKPLSLTW
PERVB      MHPTLSWRHLPTRGGEPKRLRIPLSFASIAWFLTLTITPQASSKRLIDSSNPHRPLSLTW
POEV1      MHPTLSRRHLPTRGGEPKRLRIPLSFASIAWFLTLTITPQASSKRLIDSSNPHRPLSLTW
           *****. ***  :* :****:********:*****:**.*... :: ** * *:******

PERVA      LLTDSGTGININSTQGEAPLGTWWPELYVCLRSVIPGLNDQATPPDVLRAYGFYVCPGPP
POEVMSL    LITDSGTGININNTQGEAPLGTWWPDLYVCLRSVIPSL---TSPPDILHAHGFYVCPGPP
TSUKUBA    LITDSGTGININNTQGEAPLGTWWPDLYVCLRSVIPSL---TSPPDILHAHGFYVCPGPP
PERVB      LIIDPDTGVTVNSTRGVAPRGTWWPELHFCLRLINPAVK--STPPNLVRSYGFYCCPG-T
POEV1      LIIDPDTGVTVNSTRGVAPRGTWWPELHFCLRLINPAVK--STPPNLVRSYGFYCCPG-T
           *: *..**:.::.::* **  ****:*:.*** : *.:   ::.*::: :: *  *** .

PERVA      NNEEYCGNPQDFFCKQWSCITSNDGNWKWPVSQQDRVSYSFVNNPTSYNQFNYGHGRWKD
POEVMSL    NNGKHCGNPRDFFCKQWNCVTSNDGYWKWPTSQQDRVSFSYVNTYTSSGQFNY-----LT
TSUKUBA    NNGKHCGNPRDFFCKQWNCVTSNDGYWKWPTSQQDRVSFSYVNTYTSSGQFNY-----LT
PERVB      EKEKYCGGSGESFCRRWSCVTSNDGDWKWPISLQDRVKFSFVNSG--PGKYKVMK-----
POEV1      EKEKYCGGSGESFCRRWSCVTSNDGDWKWPISLQDRVKFSFVNSG--PGKYKMMK-----
           :: :: *.. : * :: .*:*****  *** * ****.:*:**       :

PERVA      WQQRVQKDVRNKQISCHSLDLDYLKISFTE--KGKQENIQKWVNGISWGIVYYGGSGRKK
POEVMSL    W-------IRTGSPKCSPSDLDYLKISFTE--KGKQENILKWVNGMSWGMVYYGGSGKQP
TSUKUBA    W-------IRTGSPKCSPSDLDYLKISFTE--KGKQENILKWVNGMSWGMVYYGGSGKQP
PERVB      ---------LYKDKSCSPSDLDYLKISFTE--KGKQENIQKWINGMSWGIVFYKYGGG-A
POEV1      ---------LYKDKSCSPSDLDYLKISFTERKTGKYSKVDKWY---ELGNSFLLYGGG-A
                  .  * . ***********  . * .:: **    . *  :    .

PERVA      GSVLTIRLRIETQMEPPVAIGPNKGLAEQGPPIQEQRP-SPNPSD----------YNTT
POEVMSL    GSILTIRLKIN-QLEPPMAIGPNTVLTGQRPPTQ-----GPGPS----------SNIT
TSUKUBA    GSILTIRLKIN-QLEPPMAIGPNTVLTGQRPPTQ-----GPGPS----------SNIT
PERVB      GSTLTIRLRIETGTEPPVAVGPDKVLAEQGPPALEPPHNLPVPQLTSLRPDITQPPSNGT
POEV1      GSTLTIRLRIETGTEPPVAMGPDKVLAEQGPPALEPPHNLPVPQLTSLRPDITQPPSNST
           ** *****:*.   ***:*:**:. *: * .*          *.             : *

PERVA      SGSVPT-----EPNITIKTGAKLFSLIQGAFQALNSTTPEATSSCWLCLASGPPYYEGMA
POEVMSL    SGSDPT-----ESNSTTKMGAKLFSLIQGAFQALNSTTPEATSSCWLCLASGPPYYEGMA
TSUKUBA    SGSDPT-----ESSSTTKMGAKLFSLIQGAFQALNSTTPEATSSCWLCLALGPPYYEGMA
PERVB      TGLIPTNTPRNSPGVPVKTGQRLFSLIQGAFQAINSTDPDATSSCWLCLSSGPPYYEGMA
POEV1      TGLIPTNTPRNSPGVPVKTGQRLFSLIQGAFQAINSTDPDATSSCWLCLSSGPPYYEGMA
           :   *.     ... . * * :***********:*** *:*********: .****: **

PERVA      RGGKFNVTKEHRDQCTWGSQNKLTLTEVSGKGTCIGMVPPSHQHLCNHTEAFNRTSESQY
POEVMSL    RRGKFNVTKEHRDQCTWGSQNKLTLTEVSGKGTCIGKVPPSHQHLCNHTEAFNQTSESQY
TSUKUBA    RRGKFNVTKEHRDQCTWGSQNKLTLTEVSGKGTCIGKVPPSHQHLCNHTEAFNQTSESQY
PERVB      KEGKFNVTKEHRNQCTWGSRNKLTLTEVSGKGTCIGKAPPSHQHLCYSTVVYEQASENQY
POEV1      KERKFNVTKEHRNQCTWGSRNKLTLTEVSGKGTCIGKAPPSHQHLCYSTVVYEQASENQY
           :  ******:**:******:***********.***   ..******   * .::::**.**

PERVA      LVPGYDRWWACNTGLTPCVSTLVFNQTKDFCVMVQIVPRVYYYPEKAVLDEYDYRYNRPK
POEVMSL    LVPGYDRWWACNTGLTPCVSTLVFNQTKDFCIMVQIVPRVYYYPEKAILDEYDYRNHRQK
TSUKUBA    LVPGYDRWWACNTGLTPCVSTLVFNQTKDFCIMVQIVPRVYYYPEKAILDEYDYRNHRQK
PERVB      LVPGYNRWWACNTGLTPCVSTSVFNQSKDFCVMVQIVPRVYYHPEEVVLDEYDYRYNRPK
POEV1      LVPGYNRWWACNTGLTPCVSTSVFNQSKDFCVMVQIVPRVYYHPEEVVLDEYDYRYNRPK
           *****:*************** ****:*** :**********:*::.:******* :* *
```

```
PERVA       REPISLTLAVMLGLGVAAGVGTGTAALITGPQQLEKGLSNLHRIVTEDLQALEKSVSNLE
POEVMSL     REPISLTLAVMLGLGVAAGVGTGTAALVTGPQQLETGLSNLHRIVTEDLQALEKSVSNLE
TSUKUBA     REPISLTLAVMLGLGVAAGVGTGTAALVTGPQQLETGLSNLHRIVTEDLQALEKSVSNLE
PERVB       REPVSLTLAVMLGLGTAVGVGTGTAALITGPQQLEKGLGELHAAMTEDLRALEESVSNLE
POEV1       REPVSLTLAVMLGLGTAVGVGTGTAALITGPQQLEKGLGELHAAMTEDLRALKESVSNLE
            ::*:***********. .** ******:*******.**.:**  :* :*:**::******

PERVA       ESLTSLSEVVLQNRRGLDLLFLKEGGLCVALKEECCFYVDHSGAIRDSMSKLRERLERRR
POEVMSL     ESLTSLSEVVLQNRRGLDLLFLKEGGLCVALKEECCFYVDHSGAIRDSMNKLRERLEKRR
TSUKUBA     ESLTSLSEVVLQNRRGLDLLFLKEGGLCVALKEECCFYVDHSGAIRDSMNKLRERLEKRR
PERVB       ESLTSLSEVVLQNRRGLDLLFLKEGGLCVALKEECCFYVDHSGAIRDSMNKLRERLEKRR
POEV1       ESLTSLSEVVLQNRRGLDLLFLREGGLCAALKEECCFYVDHSGAIRDSMSKLRERLERRR
            :************:::*******::  **.****:***************.**:::**::*

PERVA       REREADQGWFEGWFNRSPWMTTLLSALTGPLVVLLLLLTVGPCLINRFVAFVRERVSAVQ
POEVMSL     REKETTQGWFEGWFNRSLWLATLLSALTGPLIVLLLLLTVGPCIINKLIAFIRERISAVQ
TSUKUBA     REKETTQGWFEGWFNRSPWLATLLSALTGPLIVLLLLLTVGPCIINKLIAFIRERISAVQ
PERVB       REREADQGWFEGWFNRSPWMTTLLSALTGPLVVLLLLLTVGPCLINRFVAFVRERVSAVQ
POEV1       REREADQGWFEGWFNRSPWMTTLLSALTGPLVVLLLLLTVGPCLINRFVAFVRERVSAVQ
            **:*: * **: *:*** *::****:*****:*******:***:**:::**:*:*:****

PERVA       IMVLRQQYQGLLSQGETDL
POEVMSL     IMVLRQQYQSPSSR-EAGR
TSUKUBA     IMVLRQQYQSPSSR-EAGR
PERVB       IMVLRQQYQGLLSQGETDL
POEV1       IMVLRQQYQGLLSQGETDL
            *********.  .: *:.
```

**Figure 2.** Alignment of PoERV envelope genes. The amino acid sequences are derived from the published nucleotide sequences as follows: PERV; PERV A, PERV B, PoEV 1, (Galbraith *et al.*, 1997); PERV MSL, Tsukuba (Fishman, 1997)

## FIGURE 3

## VARIABLE REGION OF PoERV ENVELOPE POLYPEPTIDE SHOWING POSITIONS OF PEPTIDES

```
PERVA      NNEEYCGNPQDFFCKQWSCITSNDGNWKWPVSQQDRVSYSFVNNPTSYNQFNYghgrwkd
POEVMSL    NNGKHCGNPRDFFCKQWNCVTSNDGYWKWPTSQQDRVSFSYVNTYTSSGQFNY-----LT
TSUKUBA    NNGKHCGNPRDFFCKQWNCVTSNDGYWKWPTSQQDRVSFSYVNTYTSSGQFNY-----LT
PERVB      EKEKYCGGSGESFCRRWSCVTSNDGDWKWPISLQDRVKFSFVNSG--PGKYKVMK-----
POEV1      EKEKYCGGSGESFCRRWSCVTSNDGDWKWPISLQDRVKFSFVNSG--PGKYKMMK-----

PERVA      wqqrvqkdvrnkqisCHSLDLDYLKISFTE--KGKQENIQKWVNGISWGIVYYGGSGRKK
POEVMSL    W--------IRTGSPKCSPSDLDYLKISFTE--KGKQENILKWVNGMSWGMVYYGGSGKQP
TSUKUBA    W--------IRTGSPKCSPSDLDYLKISFTE--KGKQENILKWVNGMSWGMVYYGGSGKQP
PERVB      ----------LYKDKSCSPSDLDYLKISFTE--kgkqeniqkwingmsWGIVFYKYGGG-A
POEV1      ----------LYKDKSCSPSDLDYLKISFTErktgkyskvdkwy---elgnsFLLYGGG-A

PERVA      GSVLTIRLRIETQMEPPVAIGPNKGLAEQGPPiqeqrp-spnpsd----------yntt
POEVMSL    GSILTIRLKIN-QLEPPMAIGPNTVLTGQRPPTQ-----GPGPS------------SNIT
TSUKUBA    GSILTIRLKIN-QLEPPMAIGPntvltgqrpptq-----GPGPS------------SNIT
PERVB      GSTLTIRLRIETGTEPPVAVGPDKVLAEQGPPALEPPHNLPVPQLTSLRPDITQPPSNGT
POEV1      GSTLTIRLRIETGTEPPVAMGPDKVLAEQGPPALEPPHNLPVPQLtslrpditqppsnst

PERVA      SGSVPT-----EPNITIKTGAKLFSLIQGAFQALNSTTPEATSSCWLCLASGPPYYEGMA
POEVMSL    SGSDPT-----ESNSTTKMGAKLFSLIQGAFQALNSTTPEATSSCWLCLASGPPYYEGMA
TSUKUBA    SGSDPT-----ESSSTTKMGAKLFSLIQGAFQALNSTTPEATSSCWLCLALGPPYYEGMA
PERVB      TGLIPTNTPRNSPGVPVKTGQRLFSLIQGAFQAINSTDPDATSSCWLCLSSGPPYYEGMA
POEV1      TGLIPTNTPRNSPGVPVKTGQRLFSLIQGAFQAINSTDPDATSSCWLCLSSGPPYYEGMA
```

**Figure 3**. The peptides D-H and J are shown in lower case bold. Exact match only is shown. Peptide D:TSLRPDITQPPSNSTT (POEV1); Peptide E:KGKQENIQKWINGMS (PERVB); Peptide F:RKTGKYSKVDKWYELGNS (POEV1); Peptide G: NTVLTGQRPPTQ (TSUKUBA); Peptide H: GHGRWKDWQQRVQKDVRNKQIS (PERVA); Peptide J: IQEQRPSPNPSDYNTT (PERVA).

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 February 2001 (22.02.2001)

PCT

(10) International Publication Number
WO 01/012816 A3

(51) International Patent Classification[7]: C12N 15/49, C07K 14/15, G01N 33/50, C07K 16/10, A61K 39/21

(21) International Application Number: PCT/GB00/03159

(22) International Filing Date: 16 August 2000 (16.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9919604.0 18 August 1999 (18.08.1999) GB

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published:**
— *with international search report*

(88) Date of publication of the international search report:
11 July 2002

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: PORCINE ENDOVIRUS GAG AND ENV AND DIAGNOSTIC USES THEREOF

MGQTVTTPLSLTLDHWTEVRSRAHNLSVQVKKGPWQTFCASEWPTFDVGWPSEGTFNSEIILAVKAIIFQ
TGPGSHPDQEPYILTWQDLAEDPPPWVKPWLNKPRKPGPRILALGEKNKHSAEKVEPSSSYLPRDRGAAD
LAGTPTCSPTPLSSTGCCEGTSAPPGAPVVEGPAAGTRSRRGATPERTDEIAILPLRTYGPPMPGGQLQP
LQYWPFSSADLYNWKTNHPPFSEDPQRLTGLVESLMFSHQPTWDDCQQLLQTLFTTEERERILLEARKNV
PGADGRPTQLQNEIDMGFPLTRPGWDYNTAEGRESLKIYRQALVAGLRGASRRPTNLAKVREVMQGPNEP
PSVFLERLMEAFRRFTPFDPTSEAQKASVALAFIGQSALDIRKKLQRLEGLQEAELRDLVREAEKVYYRR
ETEEEKEQRKEKEREEreerrdrrqeknltkILAAVVEGKSSRERERDFRKIRSGPRQSGNLGNRTPLDK
DQCAYCKEKGHWarncpkkgnkgpkvLALEEDKD

(57) Abstract: The present invention relates to polypeptide fragments derived from porcine endogenous retrovirus (PoERV) GAG and ENV polypeptides, and to their use in detection of PoERV antibodies in a test sample. Also provided are antibodies to GAG and ENV polypeptides, which may be used to detect PoERV in a sample. Polypeptide sequences are provided which are common to several strains of PoERV, as are sequences specific to a single PoERV strain.

WO 01/012816 A3

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 00/03159

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/49 C07K14/15 G01N33/50 C07K16/10 A61K39/21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | AKIYOSHI D E ET AL: "IDENTIFICATION OF A FULL-LENGTH CDNA FOR AN ENDOGENOUS RETROVIRUS OF MINIATURE SWINE" JOURNAL OF VIROLOGY, THE AMERICAN SOCIETY FOR MICROBIOLOGY, US, vol. 72, no. 5, May 1998 (1998-05), pages 4503-4507, XP001002575 ISSN: 0022-538X page 4504, column 2, paragraph 2; table 1 page 4507, paragraph 2 - paragraph 3 --- -/-- | 1-4,19 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 16 August 2001 | 31. 10. 2001 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | CHAMBONNET, F |

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | MATTHEWS A L ET AL: "DEVELOPMENT AND VALIDATION OF A WESTERN IMMUNOBLOT ASSAY FOR DETECTION OF ANTIBODIES TO PORCINE ENDOGENOUS RETROVIRUS" TRANSPLANTATION, WILLIAMS AND WILKINS, BALTIMORE, MD, US, vol. 67, no. 7, 15 April 1999 (1999-04-15), pages 939-943, XP001006259 ISSN: 0041-1337 cited in the application the whole document | 1,2, 13-16,19 |
| X | WO 97 40167 A (GALBRAITH DANIEL NORMAN ;HAWORTH CHRISTINE (GB); LEES GILLIAN MARG) 30 October 1997 (1997-10-30) cited in the application page 3; claims 13-18,27,29,30; figure 3 | 1-4,12, 19 |
| P,X | GALBRAITH DN, KELLY HT, DYKE A, REID G, HAWORTH C, BEEKMAN J, SHEPHERD A, SMITH KT.: "Design and validation of immunological tests for the detection of Porcine endogenous retrovirus in biological materials." J VIROL METHODS. 2000 NOV;90(2):115-24., XP001006236 the whole document | 1-4, 13-19 |
| P,A | WILSON C A ET AL: "EXTENDED ANALYSIS OF THE IN VITRO TROPISM OF PORCINE ENDOGENOUS RETROVIRUS" JOURNAL OF VIROLOGY, THE AMERICAN SOCIETY FOR MICROBIOLOGY, US, vol. 74, no. 1, January 2000 (2000-01), pages 49-56, XP001002577 ISSN: 0022-538X | |
| P,A | BLUSCH J H ET AL: "A POLYMERASE CHAIN REACTION-BASED PROTOCOL FOR THE DETECTION OF TRANSMISSION OF PIG ENDOGENOUS RETROVIRUSES IN PIG TO HUMAN XENOTRANSPLANTATION" TRANSPLANTATION, WILLIAMS AND WILKINS, BALTIMORE, MD, US, vol. 69, no. 10, 27 May 2000 (2000-05-27), pages 2176-2172, XP001005967 ISSN: 0041-1337 | |

# INTERNATIONAL SEARCH REPORT

...ernational application No.
PCT/GB 00/03159

## Box I Observations where certain claims wer found unsearchable (Continuation of item 1 of first sh et)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 19 is partially directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

see extra sheet invention 1.

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claim : 4 and partially 1 to 3 and 12 to 19

A porcine endogenous retrovirus (PoERV) GAG polypeptide fragment, wherein said GAG polypeptide fragment comprises the amino acid sequence SEQ ID NO 12 identified as Peptide 1; a porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises said fragment Peptide 1 and a fragment of a PoERV ENV polypeptide; an antiserum specific to a said Peptide 1; a specific antibody of fragment thereof raised against said Peptide 1 and use thereof in the detection of PoERV in a sample; uses of said Peptide 1 or said specific antibody in the detection of PoERV in a sample or in therapy ; an assay kit for use in the detection of PoERV in a sample comprising either said Peptide 1 or said specific antibody;

2. Claim : 5 and partially 1 to 3 and 12 to 19

A porcine endogenous retrovirus (PoERV) GAG polypeptide fragment, wherein said GAG polypeptide fragment comprises the amino acid sequence SEQ ID NO 13 identified as Peptide 2; a porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises said fragment Peptide 2 and a fragment of a PoERV ENV polypeptide; an antiserum specific to a said Peptide 2; a specific antibody of fragment thereof raised against said Peptide 2 and use thereof in the detection of PoERV in a sample; uses of said Peptide 2 or said specific antibody in the detection of PoERV in a sample or in therapy ; an assay kit for use in the detection of PoERV in a sample comprising either said Peptide 2 or said specific antibody;

3. Claim : Partially 1 and 6 to 19

A porcine endogenous retrovirus (PoERV) ENV polypeptide fragment, wherein said ENV polypeptide fragment comprises the amino acid sequence SEQ ID NO 14 identified as Peptide D ; a porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises said fragment Peptide D and a fragment of a PoERV GAG polypeptide; an antiserum specific to a said Peptide D; a specific antibody of fragment thereof raised against said Peptide D and use thereof in the detection of PoERV in a sample; uses of said Peptide D or said specific antibody in the detection of PoERV in a sample or in therapy ; an assay kit for use in the detection of PoERV in a sample comprising either said Peptide D or said specific antibody;

4. Claim : Partially 1 and 6 to 19

A porcine endogenous retrovirus (PoERV) ENV polypeptide fragment, wherein said ENV polypeptide fragment comprises the amino acid sequence SEQ ID NO 15 identified as Peptide E ; a porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises said fragment Peptide E and a fragment of a PoERV GAG polypeptide; an antiserum specific to a said Peptide ED; a specific antibody of fragment thereof raised against said Peptide E and use thereof in the detection of PoERV in a sample; uses of said Peptide E or said specific antibody in the detection of PoERV in a sample or in therapy ; an assay kit for use in the detection of PoERV in a sample comprising either said Peptide E or said specific antibody;

5. Claim : Partially 1 and 6 to 19

A porcine endogenous retrovirus (PoERV) ENV polypeptide fragment, wherein said ENV polypeptide fragment comprises the amino acid sequence SEQ ID NO 16 identified as Peptide F ; a porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises said fragment Peptide F and a fragment of a PoERV GAG polypeptide; an antiserum specific to a said Peptide F; a specific antibody of fragment thereof raised against said Peptide F and use thereof in the detection of PoERV in a sample; uses of said Peptide F or said specific antibody in the detection of PoERV in a sample or in therapy ; an assay kit for use in the detection of PoERV in a sample comprising either said Peptide F or said specific antibody;

6. Claim : Partially 1 and 6 to 19

A porcine endogenous retrovirus (PoERV) ENV polypeptide fragment, wherein said ENV polypeptide fragment comprises the amino acid sequence SEQ ID NO 17 identified as Peptide G ; a porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises said fragment Peptide G and a fragment of a PoERV GAG polypeptide; an antiserum specific to a said Peptide G; a specific antibody of fragment thereof raised against said Peptide G and use thereof in the detection of PoERV in a sample; uses of said Peptide G or said specific antibody in the detection of PoERV in a sample or in therapy ; an assay kit for use in the detection of PoERV in a sample comprising either said Peptide G or said specific antibody;

7. Claim : Partially 1 and 6 to 19

A porcine endogenous retrovirus (PoERV) ENV polypeptide fragment, wherein said ENV polypeptide fragment comprises the amino acid sequence SEQ ID NO 18 identified as Peptide H

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

; a porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises said fragment Peptide H and a fragment of a PoERV GAG polypeptide; an antiserum specific to a said Peptide H; a specific antibody of fragment thereof raised against said Peptide H and use thereof in the detection of PoERV in a sample; uses of said Peptide H or said specific antibody in the detection of PoERV in a sample or in therapy ; an assay kit for use in the detection of PoERV in a sample comprising either said Peptide H or said specific antibody;

8. Claim : Partially 1 and 6 to 19

A porcine endogenous retrovirus (PoERV) ENV polypeptide fragment, wherein said ENV polypeptide fragment comprises the amino acid sequence SEQ ID NO 19 identified as Peptide J ; a porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises said fragment Peptide J and a fragment of a PoERV GAG polypeptide; an antiserum specific to a said Peptide J; a specific antibody of fragment thereof raised against said Peptide J and use thereof in the detection of PoERV in a sample; uses of said Peptide J or said specific antibody in the detection of PoERV in a sample or in therapy ; an assay kit for use in the detection of PoERV in a sample comprising either said Peptide J or said specific antibody;

9. Claim : Partially 12 to 19

A porcine endogenous retrovirus (PoERV) polypeptide fragment, wherein said polypeptide fragment comprises a fragment of a PoERV GAG polypeptide and a fragment of a PoERV ENV polypeptide, as far as not covered by a previous subject; an antiserum specific to a said PoERV polypeptide; a specific antibody of fragment thereof raised against said PoERV polypeptide and use thereof in the detection of PoERV in a sample; uses of said PoERV polypeptide or said specific antibody in the detection of PoERV in a sample or in therapy ; an assay kit for use in the detection of PoERV in a sample comprising either said PoERV polypeptide or said specific antibody.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 00/03159

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 9740167 A | 30-10-1997 | AU 721579 B | 06-07-2000 |
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| | | CA 2251939 A | 30-10-1997 |
| | | EP 0907739 A | 14-04-1999 |
| | | JP 2000512129 T | 19-09-2000 |